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THE LOESS SOILS OF THE NEBRASKA PORTION OF THE TRANSITION REGION:

III. POTASH, SODA, AND PHOSPHORIC ACID.[1]

BY F. J. ALWAY, *Chief, Division of Soils, Agricultural Experiment Station, University of Minnesota,* AND R. M. ISHAM, *Chief Chemist, Research Department, Pittsburg Testing Laboratory.*[2]

INTRODUCTION.

In Nebraska the loess extends westward for about 300 miles from the eastern boundary on the Missouri River. Throughout this distance the temperature conditions are quite uniform, but there is a gradual decrease in the humidity of the climate, the normal annual precipitation, which exceeds 30 inches at the eastern boundary, steadily falling until it is less than 20 inches in the extreme western portion, while the rate of evaporation increases considerably. The climate of this region has been considered in detail in a previous paper (3).

The soil samples, upon which this article is based, were collected from 30 virgin prairie fields, 5 near each of six stations of the United States Weather Bureau shown in figure 1—Wauneta, McCook, Holdrege, Hastings, Lincoln, and Weeping Water. In each field, at intervals of 30 feet, ten borings were made to a depth of 6 feet and composite samples prepared of each foot-section, thus giving 6 samples from each field, the so-called "field samples." From these we prepared the "area samples," by mixing equal weights of the corresponding five "field samples." Thus each of the "area samples" is a composite from 50 individual borings. The details of the methods of sampling are given in the article referred to above.

[1] Received for publication February 10, 1916.

[2] The work reported in this paper was carried out during the summers of 1911 and 1912 at the Nebraska Agricultural Experiment Station, where the authors were Chemist and Assistant in Chemistry, respectively. Mr. J. W. Tobiska assisted during the latter summer.

TOTAL AMOUNTS PRESENT.

In the case of the "area samples" the total potash and soda were determined by the J. Lawrence Smith method, and the total phosphoric acid by digestion with hydrofluoric and nitric acids (12, p. 163). Also, the proportion of each of the three constituents dissolved by a five-day digestion with strong hydrochloric acid (7, p. 18) and the portion of the potash and the phosphoric acid soluble in a 1 per cent citric acid solution (5), were determined. In the case of the first-foot "field samples" from all thirty fields the total phosphoric acid was determined as above mentioned and the total potash by the modified J. Lawrence Smith method (11), which does not include the soda.

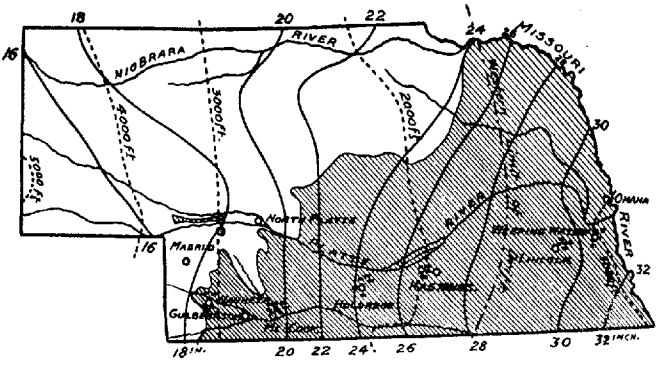

Fig. 1—Map of Nebraska showing distribution of the loess (shaded), annual precipitation and location of the fields sampled.

The total potash and soda in the area samples are reported in Table I. The distribution of potash throughout the extensive region is very uniform, whether the different depths of the same area, or the different areas are compared. While on the whole the potash content is somewhat higher in the deeper sections the variations are small and irregular. The two eastern areas show slightly lower, and the extreme western slightly higher, amounts than the intervening three. The distribution of the soda shows greater differences. In the western four areas it is quite uniform, both from the surface downward and from area to area, but in the eastern two the amount of soda is distinctly less than in the others, and also is greater in the lower than in the upper three feet.

The data reported in Table I were included in a previous article by one of the authors (1). Later, in checking over the analyses, it was found that an error had been made in introducing corrections for the soda and the potash contained in the reagents, the correction for the former having been deducted from the found percentage of the latter and vice versa. Accordingly the data in the article referred to are .07 or .08 per cent too low for potash and too high for soda. In the case of a few of the samples new determinations have been made.

TABLE I.

TOTAL POTASH AND SODA IN THE FOOT-SECTIONS FROM THE SIX AREAS.

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
			POTASH (K_2O).				
1	2.63	2.51	2.40	2.49	2.46	2.46	2.49
2	2.68	2.49	2.46	2.45	2.47	2.38	2.49
3	2.70	2.50	2.56	2.51	2.51	2.42	2.53
4	2.65	2.55	2.67	2.56	2.54	2.37	2.56
5	2.67	2.63	2.64	2.67	2.52	2.45	2.60
6	2.75	2.60	2.66	2.65	2.53	2.42	2.60
Average	2.68	2.55	2.56	2.55	2.50	2.42	2.54
			SODA (Na_2O).				
1	1.41	1.50	1.50	1.48	0.96	1.05	1.32
2	1.43	1.49	1.38	1.36	0.94	0.99	1.27
3	1.34	1.40	1.40	1.36	1.06	1.04	1.27
4	1.42	1.36	1.44	1.59	1.14	1.29	1.37
5	1.45	1.51	1.57	1.47	1.21	1.27	1.41
6	1.48	1.50	1.49	1.54	1.18	1.37	1.43
Average	1.42	1.46	1.46	1.47	1.08	1.17	1.34

In the surface foot the potash varies only little from field to field in the same area (Table II), but is slightly higher in the fields near Wauneta than in those of the more easterly areas.

TABLE II.

TOTAL POTASH IN THE SURFACE FOOT OF THE DIFFERENT FIELDS OF EACH OF THE SIX AREAS.

Field No.	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %
I	2.62	2.52	2.46	2.57	2.42	2.46
II	2.59	2.46	2.53	2.43	2.44	2.47
III	2.59	2.54	2.52	2.49	2.40	2.43
IV	2.59	2.53	2.48	2.51	2.45	2.43
V	2.60	2.56	2.47	2.61	2.44	2.43
Average	2.60	2.52	2.49	2.52	2.43	2.44

The total phosphoric acid, while showing a greater variation than the potash, is also quite evenly distributed (Table III). In the first foot and in the second it seems much the same throughout, but in the deeper sections it shows a higher content in the two eastern areas. In the surface foot-samples from the thirty fields (Table IV) it shows almost as great a uniformity as the potash except that it is somewhat lower in all the fields of the Hastings area. The data on the amounts in the first and second feet, compared with those in the lower levels of the Lincoln area, as reported in Table V, indicate that the subsoil in this area is uniformly richer in phosphoric acid than the surface soil.

TABLE III.

TOTAL PHOSPHORIC ACID IN THE FOOT-SECTIONS FROM THE SIX AREAS.

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
1	.124	.135	.140	.107	.132	.126	.127
2	.129	.122	.113	.107	.139	.115	.121
3	.116	.115	.131	.116	.160	.125	.127
4	.151	.117	.151	.108	.166	.160	.142
5	.149	.128	.130	.135	.187	.182	.152
6	.137	.129	.108	.147	.171	.171	.144
Average	.134	.124	.129	.120	.159	.146	.135

TABLE IV.

TOTAL PHOSPHORIC ACID IN THE SURFACE FOOT OF THE DIFFERENT FIELDS OF EACH OF THE SIX AREAS.

Field No.	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %
I	.139	.132	.139	.111	.130	.130
II	.131	.132	.139	.104	.146	.120
III	.125	.132	.139	.105	.140	.126
IV	.125	.126	.132	.105	.146	.126
V	.145	.139	.145	.119	.132	.132
Average	.133	.132	.139	.109	.139	.127

TABLE V.

TOTAL PHOSPHORIC ACID AT DIFFERENT DEPTHS IN THE FIVE FIELDS OF THE LINCOLN AREA.

Depth Foot	Field I %	Field II %	Field III %	Field IV %	Field V %	Average %
1	.130	.146	.140	.146	.132	.139
2	.146	.147	.146	.138	.117	.139
3 & 4	.168	.160	.161	.163	.171	.165
5 & 6	.184	.175	.165	.178	.160	.172

Hydrochloric Acid Soluble Portions.

Both potash and soda were determined by Hilgard's method of acid extraction, digesting with hydrochloric acid of 1.115 sp. gr. for 5 days over the steam bath (7, p. 18). In the same extract the phosphoric acid was determined, but it should be pointed out that this is not the method of analysis for phosphoric acid employed by Hilgard, who ignites the sample and then digests it with strong nitric acid for 2 days. The data reported on these hydrochloric acid soluble fractions (Table VI) are from single analyses, while for the total constituents we use the average of concordant duplicate determinations. In some cases the acid-soluble portion is clearly out of place, even exceeding the total. The exceptional values referred to, however, do not materially affect the results as a whole. In order to determine finer differences it would be necessary both to make duplicate determinations and to use a weight of soil greater than we employed, viz., 2 to 4 gm.

TABLE VI.

POTASH, SODA AND PHOSPHORIC ACID, IN FOOT-SECTIONS FROM THE SIX AREAS, DISSOLVED BY 5-DAY DIGESTION WITH HYDROCHLORIC ACID OF SP. GR. 1.115.

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
			POTASH.				
1	.96	1.15	1.13	1.15	1.09	1.25	1.12
2	1.17	1.23	1.35	1.42	1.14	1.42	1.29
3	1.06	1.27	1.33	1.46	1.16	1.43	1.28
4	1.14	1.22	1.36	1.36	1.26	1.37	1.28
5	1.16	1.21	1.32	1.38	1.29	1.38	1.29
6	1.16	1.22	1.32	1.35	1.27	1.37	1.28
Average	1.11	1.22	1.30	1.35	1.20	1.37	1.26
			SODA.				
1	.32	.47	.32	.48	.39	.23	.37
2	.37	.41	.42	.45	.45	.26	.39
3	.43	.49	.50	.46	.43	.33	.44
4	.33	.45	.50	.54	.51	.29	.44
5	.53	.41	.45	.46	.48	.37	.45
6	.43	.38	.47	.42	.46	.33	.41
Average	.40	.43	.44	.47	.45	.30	.42
			PHOSPHORIC ACID.				
1	.121	.121	.115	.104	.115	.105	.114
2	.119	.108	.105	.111	.119	.104	.111
3	.100	.110	.127	.118	.115	.111	.114
4	.133	.105	.137	.105	.145	.159	.131
5	.130	.105	.130	.133	.135	.159	.132
6	.124	.108	.092	.127	.140	.166	.126
Average	.121	.110	.118	.116	.128	.134	.121

The acid-soluble potash is lowest in the two western areas, reaching a minimum at Wauneta, where the total potash is highest, but where also there is the highest proportion of very fine sand. In each area the proportion soluble in acid is somewhat the lowest in the surface foot. The proportion of the total soda soluble in acid is lower, varying from 22 to 36 per cent, shows no distinct dependence upon the depth, and does not differ between the humid and the semi-arid areas. The proportion of the total phosphoric acid dissolved averages over 90 per cent, the lowest found being 82, and shows no dependence upon the depth.

Composition of the Soil Separates.

To determine whether the separates—clay, silt, etc.—from the humid eastern areas differ chemically from the corresponding ones from the western semi-arid portion, two subsoil composites were prepared and subjected to mechanical analysis. For the former we used a composite of equal weights of the third, fourth, fifth and sixth foot area samples from Lincoln, and for the latter a similar composite from Wauneta.

TABLE VII.

COMPOSITION OF SOIL SEPARATES FROM A HUMID (A) AND A SEMI-ARID SUBSOIL (B).

	Mec'l Analyses		Potash		Soda		Phos. Acid	
	A %	B %	A %	B %	A %	B %	A %	B %
Coarse to fine sand (– to 0.1 mm.)	1.94	3.25	1.46	2.83	1.25	1.67	.....	0.057
Very fine sand (0.1 to 0.05 mm.)	5.02	54.98	2.25	2.65	1.81	1.94	0.096	0.051
Silt (0.05 to 0.005 mm.).....	56.83	22.41	2.56	2.64	1.73	1.40	0.096	0.057
Clay (0.005 to –0).....	32.24	14.24	2.19	2.42	0.33	0.37	0.126	0.120
Material soluble in HCl.....	[1]1.72	[1]5.25	2.21	2.76	6.22	1.24	5.820	1.850
Original material	100.00	100.00	2.53	2.70	1.24	1.42	0.171	0.138
Portion of material dissolved by the 1% HCl	[1]1.72	[1]5.25	0.038	0.145	0.107	0.065	0.102	0.097

[1] By difference.

In order to disintegrate thoroughly the floccules in which the soil particles were cemented together by carbonates, the soil was washed on the filter with hydrochloric acid until the washings gave no test for lime or magnesia. It was then washed free of acid and separated into clay, silt, very fine sand and a coarser fraction, which included the fine, medium and coarse sands. In each case the medium sand constituted about one-fourth of this fraction, while coarse sand was practically absent. The leachings and wash waters were collected, evaporated, and analyzed for potash, soda and phosphoric acid. In the four soil separates the total potash, soda and phosphoric acid were determined by the methods described above. Table VII shows both the proportions of the different separates and the chemical composition of these.

In the humid subsoil the potash is somewhat higher in the silt, and very much lower in the coarsest fraction, than in either the clay or the very fine sand, while in the semi-arid sample it is similar in the silt and the very fine sand and higher than in the clay but lower than in the coarsest fraction. Thus the silt and the very fine sand from the western area are alike and only slightly richer in potash than the silt from the eastern area. These two fractions together have been found to constitute from 77 to 95 per cent[1] of the weight of each of the area composites, the silt predominating in all except the Wauneta samples, and the proportion of very fine sand being lowest in the eastern two areas. The coarsest fraction forms such a very small proportion of any of the loess samples that it may be ignored. In the three other groups of separates the variation in potash is comparatively slight, the clay, which contains the least, having about nine-tenths as much as the silt, which contains the most. The comparatively slight variation in the distribution of the potash over the region is in accord with the variations in mechanical composition, together with the found composition of the separates.

In both subsoils the soda was highest in the very fine sand and much the lowest in the clay, it being in the latter but slightly more than one-fourth as high as in the original material and less than one-fifth as high as in the very fine sand. These variations are extreme, compared with those found for the potash. Accordingly we should expect to find a greater variation in the distribution of soda, as is actually the case.

In the case of both subsoils the acid used to remove the carbonates dissolved out over half of the total phosphoric acid, but only a small part of the potash and soda. From the semi-arid subsoil there was dissolved about four times as much potash but only about half as much soda as from the humid subsoil. This in comparison with the data in Table VI indicates that the relative solubility of these constituents is not the same in a cold dilute, as in a hot, concentrated, mineral acid.

It should be pointed out that the composition of these separates—especially of the clay—is not to be expected to be similar to that of those obtained by the method commonly used in this country, namely, deflocculation by means of prolonged violent shaking with a very dilute ammonia solution, followed by repeated decantations of the "clay-water" and the evaporation of this along with the clay. Also, the proportions of the various separates are different from those found by the common method, the effect of the hydrochloric acid treatment being to increase the finest separate, the so-called "clay."

[1] According to analyses by Mr. C. O. Rost, by the method described in Bureau of Soils Bulletin 84 (1912). These will be reported in the next paper in the series which will appear in Soil Science, Vol. I, No. 5, May, 1916.

Citric Acid Soluble Portions.

The uniformity in the distribution of the total potash, and to a lesser degree also that of the total phosphoric acid, in these Nebraska loess soils is such that there seems to be little promise of results of interest in further such analyses. Almost the same may be said of the portions soluble in strong hydrochloric acid. The situation, however, seems entirely different when we consider only the portions soluble in weak solvents. Attention has already been called to the much greater solubility of the potash of the western subsoil, when cold 1 per cent hydrochloric acid is used. A study of the solubility in 1 per cent citric acid solution has given unexpected results. The data reported in Tables VIII, IX and X were secured by shaking 200 gm. of soil with 2,000 c.c. of 1 per cent citric acid solution at frequent intervals through 7 days (6, p. 159). The potash and the phosphoric acid were determined in 500 c.c. aliquot portions, corresponding to 50 gm. of soil. A difference of .001 per cent of P_2O_5 or K_2O would be indicated by .008 gm. magnesium pyrophosphate or .0026 gm. potassium platinochloride, thus giving some actual significance to figures in the third decimal place. The duplicate digestions usually give a difference of less than .001 per cent, and none was accepted when this exceeded .002 per cent P_2O_5 or K_2O. The irregular intervals which characterize shaking by hand seem to exert no distinct effect upon the amount dissolved, the duplicate digestions giving as concordant results when made at intervals of several weeks as when carried on side by side; also the results obtained by two analysts, the one working a year after the other, were strictly concordant. In the later work the tedious shaking by hand was replaced by the use of a machine. Seven days' shaking by hand gave the same results as five hours in the machine; eight hours with the latter gave no higher result than five, but three gave lower. The data reported in Table XIII and part of those in XI were obtained by using the five-hour agitation.

The citric acid soluble phosphoric acid and potash in the different foot sections are reported in Table VIII. The potash increases from east to west, being lowest in the two eastern areas and highest in the extreme western. In the former it decreases somewhat in passing from the first to the sixth foot, while in the latter it distinctly increases. The intervening three areas show an intermediate behavior. The distribution of the citric acid soluble potash agrees with neither that of the total amount nor with that of the part soluble in hot strong hydrochloric acid. The increase in the solubility from east to west, and, in the western areas, from the surface downward, accompanies an increase in the carbonate content which must serve to neutralize a portion of the acid and so to lessen its solvent action. Thus in the Wauneta area the carbon dioxide content of the six area-composites, ranged in order from the first to the

sixth foot, are: 0.09, 0.07, 0.59, 1.67, 1.78 and 1.68 per cent. The carbonate contained in 200 gm. of the sixth foot sample would be sufficient to neutralize just about half of the citric acid contained in 2,000 c.c. of the solution used.

TABLE VIII.
POTASH AND PHOSPHORIC ACID SOLUBLE IN 1 PER CENT CITRIC ACID SOLUTION.

POTASH (K_2O).

Depth Foot	Wauneta %	McCook %	Holdrege %	Hastings %	Lincoln %	Wpg.Wtr. %	Average %
1	.047	.044	.048	.044	.032	.036	.042
2	.058	.055	.049	.042	.025	.032	.043
3	.073	.062	.057	.046	.021	.025	.047
4	.083	.066	.061	.049	.020	.023	.050
5	.084	.068	.064	.051	.019	.016	.050
6	.083	.067	.067	.053	.020	.016	.051
Average	.071	.060	.058	.047	.023	.025	.047

PHOSPHORIC ACID (P_2O_5).

1	.043	.040	.038	.021	.012	.010	.027
2	.045	.030	.044	.025	.016	.009	.028
3	.032	.029	.060	.047	.036	.025	.038
4	.029	.027	.050	.056	.046	.045	.042
5	.026	.026	.044	.057	.050	.057	.043
6	.026	.029	.041	.055	.055	.064	.045
Average	.033	.030	.046	.043	.036	.035	.037

To ascertain whether all the fields of the eastern areas are characterized by a greater amount of soluble potash in the upper layers of the soil, and those of the western areas by the reverse, determinations were made for the first and sixth foot samples from all the fields of the two extreme areas—Weeping Water and Wauneta (Table IX). In the former all the fields show an excess of potash in the first foot, it being about twice as great as in the sixth, while in the latter all show less in the first foot, in which it is, in general, about two-thirds as high as in the sixth.

TABLE IX.
CITRIC ACID SOLUBLE POTASH IN THE DIFFERENT FIELDS OF THE OUTER AREAS.

	Weeping Water			Wauneta		
Field No.	First Foot %	Sixth Foot %	Ratio[1] %	First Foot %	Sixth Foot %	Ratio[1] %
I	.031	.017	55	.057	.080	140
II	.034	.015	44	.051	.082	161
III	.039	.016	41	.043	.091	212
IV	.029	.016	55	.052	.078	150
V	.035	.015	43	.050	.077	154
Average	.034	.016	48	.051	.082	163

[1] The percentage in the first foot = 100.

In contrast with the potash the citric acid soluble phosphoric acid, considering the amount in the whole six-foot layer, does not increase from east to west, but is similar in the extreme areas, with the maximum in the intermediate. In the surface two feet it increases from east to west, while in the fifth and sixth it decreases. In the more easterly areas it shows an increase from the surface downward, while in the most westerly area it decreases markedly. The data for the first and the sixth foot sections from the fields of the outer areas (Table X) indicate that this difference is an area characteristic.

TABLE X.

CITRIC ACID SOLUBLE PHOSPHORIC ACID IN DIFFERENT FIELDS OF THE OUTER AREAS.

	Weeping Water			Wauneta		
Field No.	First Foot %	Sixth Foot %	Ratio[1] %	First Foot %	Sixth Foot %	Ratio[1] %
I	.009	.049	544	.030	.026	87
II	.008	.050	625	.036	.031	87
III	.013	.053	408	.046	.028	61
IV	.013	....	...	.036	.028	77
V	.011	....	...	.034	.025	74
Average	.011	....	...	.036	.028	77

[1] The percentage in the first foot = 100.

Thus we find that the citric acid soluble potash and phosphoric acid show very marked differences according to the depth of the soil layer, in the most easterly areas the former decreasing and the latter increasing, while in the most westerly area the conditions are reversed (fig. 2).

It was evident that the decrease in the amount of citric acid soluble phosphoric acid in the western areas might be due to the greater amount of carbonates in the lower sections. Hilgard (8, p. 339) has suggested that in using the citric acid extraction one make "allowance for such neutralization as may occur in the soil," but Hall (6, p. 160) considers that in attempting to establish the amount of immediately available plant food "no attempt should be made to add an extra amount of citric acid to combine with the carbonate; secondary solvent actions are set up both by the carbon dioxide evolved and by the calcium citrate formed; moreover, the real comparative basis of the method of analysis is destroyed."

A bulk sample of loess subsoil taken at a depth of from 4 to 7 feet from the Experiment Station farm was subjected to eight successive extractions. After each extraction 1,000 c.c. of solution was removed for analysis and the remainder of the clear liquid decanted. In order to remove the citric acid solution remaining mixed with the soil the bottles were filled with water, shaken vigorously, and allowed to stand until the liquid became clear, when it was decanted, this operation being repeated

four or five times. Then there was added 20 gm. of citric acid and enough water to bring the liquid up to the mark at which it had first stood after adding the 2,000 c.c. citric acid solution to the 200 gm. of soil. The successive extractions removed gradually decreasing amounts

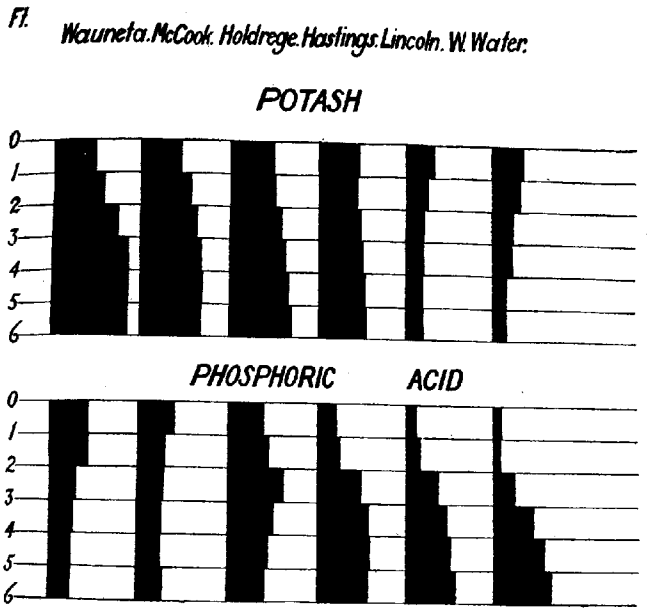

Fig. 2—Diagram showing relative amounts of citric acid soluble potash and phosphoric acid at different depths.

of phosphoric acid, this decreasing rapidly with the first three and slowly with the later ones, as shown in Table XI.

The depressing effect of the presence of calcium carbonate is shown by lines 5 and 6 in the above table. Some of the subsoil was in the one case mixed with 2 per cent and in the other with 6 per cent of calcium carbonate. Both of these were extracted at the same time as the untreated subsoil. Only .036 and .017 per cent, respectively, were dissolved from the first and second, while the last gave .061 per cent.

The first and the sixth foot samples from the fields of the outer areas, reported in Table X, were extracted a second time with citric acid, according to the method described above. As shown in Table XII, considerably less phosphoric acid was removed in the second extraction than in the first, except in the case of the calcareous subsoils of the Wauneta

area. In the latter area the two extractions together removed an average of 0.051 per cent from the first foot and 0.056 from the sixth, evidence that the decrease in solubility with increasing depth is to be attributed to the increase in the carbonate content and that such a decrease would not

TABLE XI.

AMOUNTS OF PHOSPHORIC ACID DISSOLVED BY 1 PER CENT CITRIC ACID SOLUTION, UNDER DIFFERENT CONDITIONS, FROM A SUBSOIL (4 TO 7 FEET) FROM THE LINCOLN AREA.

	Phosphoric Acid %
1. Shaken by hand 7 days	.061
2. Shaken in machine 3 hours	.056
3. Shaken in machine 5 hours	.060
4. Shaken in machine 8 hours	.061
5. After addition of 2% $CaCO_3$, shaken by hand 7 days	.036
6. After addition of 6% $CaCO_3$, shaken by hand 7 days	.017
7. Repeatedly extracted with fresh solution, the mixture being shaken 3 hours in the machine each time after the first extraction.	
1st extraction	.061
2nd extraction	.036
3rd extraction	.014
4th extraction	.008
5th extraction	.004
6th extraction	.004
7th extraction	.003
8th extraction	.002
Total removed by the 8 extractions	.132

have been found had we acted upon Hilgard's suggestion to allow for the neutralizing action of the carbonates.

TABLE XII.

RELATION OF THE AMOUNT OF PHOSPHORIC ACID REMOVED BY THE SECOND EXTRACTION WITH CITRIC ACID TO THAT REMOVED BY THE FIRST.

	First Foot			Sixth Foot		
Field No.	1st Ext. %	2nd Ext. %	Ratio[1] %	1st. Ext. %	2nd Ext. %	Ratio[1] %
WEEPING WATER.						
I	.009	.008	90	.049	.036	73
II	.008	....	..	.050	.034	68
III	.013	.006	46	.053	.038	72
IV	.013	.006	46	....	....	..
V	.011	.006	55	....	....	..
WAUNETA.						
I	.030	.021	70	.026	.020	77
II	.036	.012	33	.031	.039	126
III	.046	.016	35	.028	.032	114
IV	.036	.011	31	.028	.030	107
V	.034	.017	50	.025	.019	76
Average	.036	.015	44	.028	.028	100

[1] The percentage in the first extraction = 100.

In the case of Fields I and III of the Weeping Water area, the only ones for which the data are complete, the first extraction removed 4.6 times as much phosphoric acid from the sixth as from the first foot, and the two extractions together 4.9 times as much. Thus the greater solubility of the phosphoric acid in the lower levels of the eastern area does not become any the less striking when a second extraction is employed.

It has been pointed out that in the case of the eastern areas the citric acid soluble phosphoric acid increases rapidly as we pass from the first to the sixth foot. To ascertain whether this increase is continued below the latter depth determinations were made of several deep samples which had been collected in connection with soil moisture studies (Table XIII). All, except Nos. 12 to 19, were from fields either on the Nebraska Experiment Station farm or not more than a mile distant, the loess on all varying from 15 to 25 feet in depth and overlying Kansan till. Nos. 12 to 18 are from a farm near Elgin, Nebraska, Nos. 17 and 18 being from an alfalfa field in which the loess deposit was found to have a thickness of 35 feet and in which the roots of the alfalfa plants had developed so freely to 30 feet that the subsoil to this depth had been practically exhausted of its available water (2, p. 118). No 19 is from a deep railway cut near Blair, Nebraska, where 25 feet of loess was removed over twenty years ago. The exposed loess subsoil has since been cropped. We do not know the depth of the loess at this place, but the deposit probably extends to at least 25 feet below the present surface, or 50 feet below the original. In general the high content of citric acid soluble phosphoric acid found in the sixth foot is continued into the deeper portions of the loess but without any marked increase. The low percentages in Nos. 11, 16, 17 and 18 seem directly connected with the presence of comparatively large amounts of carbonates.

Cameron (4, p. 77) estimates that the potash and phosphoric acid annually brought to the surface soil by capillary water is more than sufficient to replace the amounts that would be removed by "one ton per acre of dry crop containing 1 per cent potash and 0.6 per cent phosphoric acid." Where, as is the case with the soils of this study, no crop had been removed since the loess was laid down, we should expect to find a concentration of these nutrients in a readily soluble form in the surface soil. However, in the humid eastern areas we actually find a great exhaustion of phosphoric acid accompanying a distinct concentration of potash, while in the most arid areas of the west there is less readily soluble potash in the surface soil than in the lower levels, and the apparent concentration of phosphoric acid in the former is due to the neutralizing action of the carbonates in the latter.

In connection with the analytical data reported above it may be of interest to mention that, as the result of field observations and pot experiments on the loess soils of this region, we have concluded that alfalfa thrives almost as well upon the exposed loess subsoils of Eastern Nebraska as upon the surface soils. The former contain from 0.04 to 0.05 per cent nitrogen as compared with 0.20 to 0.30 in the latter. The "rawness" commonly attributed to humid subsoils must be entirely wanting, at least in so far as phosphoric acid and potash are concerned.

TABLE XIII.

CITRIC ACID SOLUBLE PHOSPHORIC ACID IN THE LOESS AT DEPTHS GREATER THAN SIX FEET.

Series No.	Location	Depth Foot	Composite from	Phosph'c Acid %
1	Lincoln	7-12	From excavation	0.073
2	Experiment Station Farm	7-10	From 2 borings	0.066
3	Near above farm	6-11	From 1 boring	0.067
4	Same boring as No. 3	12-15	From 1 boring	0.044
5	Same field as No. 3	6-11	From 1 boring	0.069
6	Same boring as No. 5	12-15	From 1 boring	0.066
7	University Place	7-12	From 1 boring	0.067
8	Same boring as No. 7	13-15	From 1 boring	0.075
9	Same field as No. 7	7-12	From 1 boring	0.057
10	Same boring as No. 9	13-15	From 1 boring	0.070
11	University Place	7-18	From 2 borings	0.038
12	Elgin	1- 3	From 3 borings	0.015
13	Same boring as No. 12	4- 6	From 3 borings	0.044
14	Same boring as No. 12	7- 9	From 3 borings	0.040
15	Same farm as No. 12	7- 9	From 3 borings	0.047
16	Same boring as No. 15	10-12	From 3 borings	0.024
17	Same farm as No. 12	15-20	From new well	0.027
18	Same well as No. 17	21	From bottom of same well.	0.022
19	Blair	25	Exposed subsoil	0.057

Alfalfa probably makes a heavier draft upon the phosphoric acid than any other crop grown upon these soils. On one of the fields of this Experiment Station farm, which had been under cultivation a little over 40 years, alfalfa was grown from 1895 to 1907, all of it being removed as hay, giving an average yield of about 4 tons to the acre. No phosphate fertilizers, and little, if any, manure had been applied to the field. In 1912 composite samples were taken from each of the first six feet, 15 borings being used, and the citric acid soluble phosphoric acid was determined. The following percentages were found: 1st, 0.024; 2nd, 0.021; 3rd, 0.037; 4th, 0.041; 5th, 0.048; 6th, 0.075; average, 0.041. The first foot and the second show a considerably higher proportion than the average for the first and second foot of the five prairie fields of the Lincoln area, while for the other four feet the differences are not marked. In the alfalfa hay there was probably removed about 680 pounds of phosphoric acid and in the other crops about 450 pounds. The total, 1,130 pounds, would correspond to 0.037 per cent of the weight of an acre foot

of soil, assuming the latter to be 3,000,000 pounds, or more than the total amount of citric acid soluble phosphoric acid found in the first and second feet of the prairie fields. This affords a certain amount of support to the assumption that the plants have freely drawn for their supply upon the lower levels of the subsoil whenever their root distribution and the moisture conditions have permitted.

Comparison With Chernozem and Arid Soils.

The Chernozem soils (10, p. 326) show a content of about 2.00 per cent total potash with small variations in both directions, while from 0.4 to 1.0 per cent is dissolved by 10 hours' digestion with 10 per cent hydrochloric acid on the water bath. The total soda is about 1.00 per cent, approximately half that of the potash, but a much smaller proportion of it is soluble in hydrochloric acid.

In the Nebraska loess soils the total potash and soda are somewhat higher, averaging 2.54 and 1.36 per cent, respectively, but bear about the same relation to one another. Digestion with hydrochloric acid of specific gravity 1.115 for 120 hours dissolved an average of 1.26 per cent potash and .42 per cent soda, the latter being here also the less soluble. The data on the acid soluble portions are not directly comparable, as the digestion of the Chernozem soils was made with a weaker acid and for a much shorter period.

The total phosphoric acid content of the Chernozem soils (10, p. 327) varies from .05 to occasionally more than .30 per cent, but usually the amounts lie between .15 and .30 per cent, the acid soluble portion constituting about four-fifths. This is appreciably higher than in the Nebraska loess soils, which show an average of .127 for the surface foot and of .135 for the first six feet, with a variation, in the case of very composite samples, between .104 to .184 per cent. In the Chernozem soils the phosphoric acid of the immediate surface (4 to 8 inches) is somewhat higher than in the underlying layers. The same has been found to be true for the Nebraska loess soils when different sections of the first foot are compared.[1]

The transition soils, while in general somewhat richer in total potash and slightly poorer in phosphoric acid, may on the whole be considered as very similar to the Russian Chernozem in potash, soda and phosphoric acid.

The data on the zeolithic (acid soluble) portions are directly comparable with those reported by Hilgard (9, p. 424), in a comparison of arid and humid soils. He finds the acid-soluble potash and soda to be much higher in arid than in humid soils, while the phosphoric acid in the one is similar to that of the other. The comparison is shown in Table XIV,

[1] Unpublished data of Alway, F. J., and Rost, C. O.

where the data for the surface foot samples from the Nebraska loess are reported. The soils from all the areas resemble arid soils, those from the most easterly as much as those from the western, semi-arid areas.

TABLE XIV.

COMPARISON OF THE TRANSITION SOILS WITH ARID AND WITH HUMID SOILS IN REGARD TO ACID-SOLUBLE POTASH, SODA AND PHOSPHORIC ACID.

	Potash %	Soda %	Phos. Acid %
Arid soils. Average of 313 soils (Hilgard)	.73	.26	.12
Humid soils. Average of 466 soils (Hilgard)	.22	.09	.11
Western two areas (from 10 fields)	1.05	.40	.12
Intermediate two areas (from 10 fields)	1.14	.40	.10
Eastern two areas (from 10 fields)	1.17	.31	.11

SUMMARY.

The soils studied represent the first six foot-sections from five virgin prairie fields in each of six so-called "areas" in Nebraska, located between the Missouri River and the western limit of the loess, a distance of more than 300 miles, in which, while the temperature conditions, wind velocity and relative humidity are quite uniform, there is a great range in aridity, the annual precipitation decreasing from more than 30 inches in the east to less than 20 in the west, while the relative aridity exhibits a still greater range on account of the increase in the rate of evaporation which accompanies the decrease in precipitation.

The total potash is very uniform in distribution both from east to west and from the first to the sixth foot. While, on the whole, it is slightly lower in the eastern areas and in the higher levels, the variations are small and irregular. The proportion soluble in hot, strong hydrochloric acid seems largely dependent upon the amount of silt present, it being lowest in the most westerly area, in which, while the total potash is highest, the proportion of very fine sand also reaches its maximum.

The total soda shows somewhat more variation. In the western four areas it is quite uniformly distributed, both from area to area and from the surface downward, amounting, in general, to a little more than half as much as the total potash. In the two eastern areas it is distinctly lower; less is found in the upper than in the lower three feet, and in general it amounts to a little less than half as much as the total potash. The proportion of soda soluble in strong hydrochloric acid is lower than in the case of potash and is quite uniform.

The total phosphoric acid is still less evenly distributed. In the first two feet it seems much the same from east to west, while in the two eastern areas it is higher in amount in the lower than in the upper sec-

tions. Most of it is soluble in strong hydrochloric acid, neither location nor depth seeming to influence the proportion.

The loess consists chiefly of silt and very fine sand, with less than 5 per cent coarser than the latter. Determinations were made of the total potash, soda and phosphoric acid, as well as of the portions of these soluble in cold 1 per cent hydrochloric acid, in four separates—clay, silt, very fine sand, and coarser particles—from typical humid and semi-arid subsoils. In the very fine sand from the humid subsoil the amount of potash was found to be about the same as in the clay, but distinctly lower than in the silt. In the semi-arid subsoil it was similar in the silt and very fine sand, in both of which it was only very slightly higher than in the silt from the humid area, but was somewhat lower in the clay. In both subsoils the amount of soda was highest in the very fine sand and much the lowest in the clay. The dilute acid dissolved about four times as much potash, but only about half as much soda, from the semi-arid as from the humid subsoil, but the soluble portions of both form only a small proportion of the total amounts present. On the other hand, the dilute acid removed from both more than half the total phosphoric acid, the proportion dissolved being higher in the semi-arid subsoil. In the separates much more phosphoric acid was found in the clay than in the coarser fractions, the silt and the very fine sand, in which it was alike.

The most noteworthy differences were shown by treatment with citric acid solution. The potash soluble in this reagent was found to increase with the aridity; in the most humid areas it decreases from the surface downward, while in the least humid it increases, notwithstanding an accompanying increase in the carbonate content, which lessens the solvent action of the acid. In contrast with this, the citric acid soluble phosphoric acid was found not to increase with the aridity, when we consider the whole six-foot section; in the first two feet it increases, but in the lower four it decreases from east to west. In the most humid areas it increases rapidly from the surface to a depth of 6 feet, while in the most westerly areas it decreases. However, in the latter the difference is to be attributed to the increase in carbonate content, because when this is neutralized the sixth foot yields as much to the acid as does the first. The high content of citric acid soluble phosphoric acid is not confined to the lower portion of the six-foot sections, but continues to more than twice this depth. This high proportion of available phosphoric acid in the deep subsoil suggests a possible basis for a crop rotation in which deep-rooted legumes would provide both for the fixation of atmospheric nitrogen and for a transfer of phosphoric acid from the deep subsoil, through the medium of the plant parts, to the upper soil layers, in which the available portion is low and upon which the roots of annual and most perennial crop plants are dependent for their mineral nutrients.

In content of potash, soda and phosphoric acid the soils from all the areas resemble the Chernozem soils of Russia and the arid soils of California.

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SOME FACTORS THAT INFLUENCE NITRATE FORMATION IN ACID SOILS.[1]

BY

E. B. FRED, *Agricultural Bacteriologist,* AND E. J. GRAUL, *Instructor in Soils, Agricultural Experiment Station, University of Wisconsin.*

In earlier reports from this station, data were presented to show the formation and distribution of nitrates in cultivated soils (5, 6). This work was confined largely to a study of the distribution of nitrates in the soil and rate of nitrification throughout the growing period. No consideration was taken of the relation between soil reaction and the formation of nitrate nitrogen. Because of the large area of acid soil in the state of Wisconsin, studies of nitrification in certain types of acid soil have been undertaken.

The results of experiments made at Rothamsted show that nitrification is greatly diminished in soil rendered acid from manuring with ammonium chloride and sulphate (4). It appears that the number of nitrate-forming organisms is smaller in acid soils. Although it is reported that nitrification cannot take place in an acid medium, data from various sources show that this process is active in acid soil. For example, Temple of the Georgia Experiment Station reports that nitrification is active in a soil that requires more than 5,000 pounds of calcium carbonate per acre (10).

The following phases of nitrification were investigated: first, a study of the occurrence of nitrate-forming bacteria in acid soils and their relation to the organisms commonly found in neutral soils; second, a comparison of nitrification of organic and inorganic substances in acid and neutral soils; third, a comparison of the effect of calcium carbonate on ammonification and nitrification of organic substances.

Four types, representative of large areas of soil, were chosen. The control or neutral soil studied in connection with the acid soils was taken from the Station Farm. The soils were classified as follows: neutral Miami silt loam from Madison, acid Plainfield sand from Sparta, acid Colby silt loam from Marshfield, and acid peat from Wyeville. The reactions of the acid soils were as follows: Plainfield sand required 13,050

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pounds of calcium carbonate to neutralize one acre of soil 6 inches deep; Colby silt loam, 20,420 pounds, and Wyeville peat, 5,985 pounds[1].

In order to prevent contamination, the soils were collected in large 8-gallon sterilized cans. After removing vegetation, the sample of soil was taken to a depth of 8 inches. The soil was then expressed to the Station Laboratory and prepared for experimental study.

Depending on the nature of the experiment, the soils were weighed into sterilized flasks, tumblers, or earthenware jars. With a few exceptions, the experiments were carried out at 60 per cent saturation and at a constant temperature of 25°C. Ammonia determinations were made by distilling with magnesium oxide in copper flasks; and nitrate determinations by the phenol-sulphonic acid method. In the case of soils high in organic matter or very rich in nitrates, the results from colorimetric analyses were compared with those obtained by the reduction method. All results are expressed as milligrams of nitrogen, per 100 grams of dry soil whether present as nitrate or ammonia.

Occurrence of Nitrifying Bacteria in Acid and Non-Acid Soils.

In Solution.—The presence of nitrifying bacteria may be noted from the products formed in certain inorganic solutions. The Omelianski solutions used in this study are designed to favor the growth of these highly specialized organisms. For the nitrite bacteria, the nitrogen was added as ammonium sulphate; for the nitrate bacteria, it was added as sodium nitrite. The solutions were prepared, 25 c.c. portions in 300 c.c. Erlenmeyer flasks, and inoculated with 1-gm. samples of the various soils. The progress of the cultures was measured at regular intervals by qualitative tests. The following reagents were used: Nessler, Trommsdorf, and Diphenylamine. Table I contains the results of these tests.

Thirty days after inoculation, the Miami and Colby silt loams showed no ammonia, but a strong nitrite reaction. The conversion of nitrite to nitrate takes place even more rapidly. Twenty days after inoculation, all of the nitrite had disappeared and a strong reaction for nitrate was noted. In the case of acid sand and peat, oxidation was much slower. Enrichment cultures made by transferring a loop of the active cultures to new media gave similar results. Apparently the four types of soil contain the nitrifying organisms. When inoculated into a suitable culture medium, Miami or Colby silt loam soil nitrify much more rapidly than the acid sand or peat. The slowness of nitrification in the latter may be due to many factors, e.g., small number of organisms, decreased physiological efficiency. These factors will be discussed later.

[1] Acidity determinations in sand and Colby soil were made according to the Truog Barium-hydroxide method (11); in peat according to the Veitch method.

In Soil.—The work was arranged to include not only investigations concerning the occurrence of the nitrifying organisms in acid soils, but also their activity when transferred to a sterilized soil of a different type. The bacteria from acid soils were transplanted to a non-acid soil and *vice versa.* In this way, an attempt was made to study the nitrifying flora of acid soils as compared with those of non-acid soils. The question naturally suggested itself: Are the nitrifying bacteria commonly found in acid soils more resistant to acidity than the same group of organisms from a non-acid soil? With this in mind, the following studies were made. Because of the similarity of these tests they are discussed in one group.

TABLE I.

NITRIFICATION IN OMELIANSKI'S SOLUTION.

A.—NITRITE FORMATION.

Time in days	Uninoculated		Soil							
			Miami		Sand		Colby		Peat	
	NH_3	NO_2	NH_3	NO_2	NH_3	NO_2	NH_3	NO_2	NH_3	NO_2
10	+	—	+	—	+	—	+	—	+	—
20	+	—	+	+	+	tr	—	+	+	tr
30	+	—	—	+	+	tr	—	+	+	tr

B.—NITRATE FORMATION.

Time in days	Uninoculated		Soil							
			Miami		Sand		Colby		Peat	
	NO_2	NO_3	NO_2	NO_3	NO_2	NO_3	NO_2	NO_3	NO_2	NO_3
10	+	—	+	—	+	—	+	—	+	—
20	+	—	—	+	+	tr	—	+	+	tr
30	+	—	—	+	+	tr	—	+	+	tr

— = no reaction. + = distinct reaction. tr = trace.

Two-hundred-gram portions of the soil were weighed into 1-liter Erlenmeyer flasks, the moisture adjusted to 60 per cent saturation, and the soil heated in the autoclave for 1 hour at 15 pounds pressure. When cool, the sterile nitrogenous substances, ammonium sulphate and casein, were added in liquid form. The added nitrogen varied in the different experiments between 15 and 21 mg. per 100 gm. of soil. An exception to this is recorded in Table V. Here larger amounts were used. In each case the inoculum represented 5 gm. of soil. Each soil type was used as a medium and inoculated with the various soils. For example, Miami soil was used as a medium and inoculated with Miami, with Sparta sand, with Colby, and with peat. All determinations were made in duplicate and the averages given in the tables. The results for each soil are presented in Tables II to V.

Miami soil.—The data in Table II show, with one exception, that regardless of the source of inoculum, nitrogen from ammonium sulphate in neutral Miami soil nitrifies more rapidly than the same amount of nitrogen from casein. Within the time limit of this experiment, the acid Sparta sand and peat failed to nitrify. This agrees with the results of the test in solution.

It is apparent from the data that the activity of the nitrate-forming organisms in Miami soil is not very different from that of similar organisms in Colby soil. After 8 weeks the organisms from Colby oxidized the nitrogen of ammonium sulphate somewhat faster than those from Miami soil itself. Toward casein, the organisms from these two soils behaved alike. Evidently the nitrate-forming flora of acid Colby soil is not inferior to that of the neutral Miami silt loam. When inoculated into a neutral soil, their power to form nitrates is as great as that of the original flora.

TABLE II.

NITRIFICATION IN STERILIZED MIAMI SILT LOAM.

September 10—October 8—November 7.

Culture	Inocula	Treatment per 100 gm. of soil	Nitrate Nitrogen per 100 gm. of soil Average of duplicate flasks						
			Amt. in control	After		Increase		Nitrogen rec'd as nitrate	
				4 wks.	8 wks.	4 wks.	8 wks.	4 wks.	8 wks
		Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	%	%
1	Miami	15 N from $(NH_4)_2SO_4$..	3.9	12.1	14.0	8.2	10.1	54.6	67.3
2	Sand	15 N from $(NH_4)_2SO_4$..	3.9	3.7	3.7	...	....	....	....
3	Colby	15 N from $(NH_4)_2SO_4$..	3.9	11.6	14.74	7.7	10.84	51.3	72.0
4	Peat	15 N from $(NH_4)_2SO_4$..	3.9	....	....	...	....	....	....
5	Miami	15 N from casein.....	3.9	12.5	11.4	8.6	7.5	57.3	50.0
6	Sand	15 N from casein.....	3.9	3.5	3.57	...	....	....	....
7	Colby	15 N from casein.....	3.9	10.8	11.36	6.9	7.4	43.3	49.7
8	Peat	15 N from casein.....	3.9	....	....	...	...	....	....

Sparta sand.—The apparent absence of nitrification in Sparta sand may be due to one or more factors. First, the proper organisms may not be present; second, the conditions necessary for nitrification may not be present. If the former statement is true, then sterilized Sparta sand inoculated with the proper organisms should nitrify. Accordingly an experiment was made with Sparta sand. Here acid sand was used as a medium and inoculated with soil suspension from all of the soil types.

Nitrate tests after 8 weeks did not show any increase beyond that of the uninoculated control. The results clearly indicate that sterilized Sparta sand is not suitable for a rapid oxidation of ammonia. The evidence shows that the absence of nitrification in Sparta sand is not due entirely to lack of proper organisms. This experiment was repeated using

0.5227 gm. of calcium carbonate per 100 gm. of soil, enough to neutralize all of the active soil acidity. The results should answer the question: Does calcium carbonate produce favorable conditions for nitrification in acid Sparta sand? The data in Table III give an answer to this question.

It is realized that the technique employed is far from perfect and that sterilization of soil will no doubt seriously affect the process of nitrification. Prior to this study, acidity tests were made in order to measure the effect of heat on reaction. No decided change in reaction was noted.

From the figures in Table III it will be seen that calcium carbonate only partially corrects the adverse conditions for nitrification in sterilized Sparta sand. When sterile Sparta sand was reinoculated with organisms from sand or peat, no increase in the formation of nitrates occurred. The bacteria from Miami and Colby silt loam soils gave a slight increase in nitrates. It is evident from the data, that Sparta acid sand is not well adapted for nitrification of ammonium sulphate or casein.

TABLE III.

NITRIFICATION IN STERILIZED SAND PLUS CALCIUM CARBONATE.

November 25—December 28.

Culture	Inoculum	Treatment per 100 gm. of soil	Nitrate nitrogen per 100 gm. dry soil			
			Amount in control	After 4 weeks	Increase	Nitrogen recovered as nitrate
		Mg.	Mg.	Mg.	Mg.	%
1	Miami	14.2 N from $(NH_4)_2SO_4$	1.2	3.28	2.08	14.6
2	Sand	14.2 N from $(NH_4)_2SO_4$	0.4	....	....	....
3	Colby	14.2 N from $(NH_4)_2SO_4$	0.6	1.80	1.20	8.45
4	Peat	14.2 N from $(NH_4)_2SO_4$	0.3	....	....	....
5	Miami	14.2 N from casein....	1.2	.66	....	....
6	Sand	14.2 N from casein....	0.4	....	....	....
7	Colby	14.2 N from casein....	0.6	1.01	.41	2.8
8	Peat	14.2 N from casein....	0.3	....	....	....

Colby silt loam.—In Table IV are given the quantities of nitrate produced by bacteria from the various soil types when used to inoculate Colby silt loam. A study of the effect of calcium carbonate on nitrate formation was included. In Group B enough calcium carbonate was added to neutralize all soil acidity.

The figures of Table IV show the nitrate content after 9 weeks. In the ammonium sulphate series without calcium carbonate, there was no increase in nitrates except where inoculated with Miami soil, while in the casein series there was a decided gain in nitrates when inoculated with Miami. It is clear from the data, that inoculations from neutral Miami soil into Colby soil medium caused a great gain in nitrate nitrogen. When Colby soil was used to inoculate Colby, no formation of nitrates was noted except in the limed series. Here, the organisms from Colby behaved much the same as those from Miami.

In spite of wide variations between duplicates, it seems safe to conclude that the nitrifying bacteria from acid soils when inoculated into acid soils are not any more efficient in forming nitrates than the nitrifying bacteria from a non-acid soil. In regard to the nature of the nitrifiable substance in acid soil, organic nitrogen is oxidized to nitrates more rapidly than inorganic nitrogen (2, 10).

TABLE IV.

RATE OF NITRIFICATION IN STERILIZED COLBY SILT LOAM.

November 25—January 30.

GROUP A.—UNLIMED SERIES.

Culture	Inoculum	Treatment per 100 gm. of soil	Nitrate nitrogen per 25 gm. dry soil			
			Amount in control	After 9 weeks	Increase	Nitrogen recovered as nitrate
		Mg.	Mg.	Mg.	Mg.	%
1	Miami	14.2 N from $(NH_4)_2SO_4$	2.92	4.05	1.13	7.9
2	Sand	14.2 N from $(NH_4)_2SO_4$	3.42	2.80	....	....
3	Colby	14.2 N from $(NH_4)_2SO_4$	3.40	2.97	....	....
4	Peat	14.2 N from $(NH_4)_2SO_4$	3.08	2.76	....	....
5	Miami	14.2 N from casein....	2.92	6.42	3.50	24.6
6	Sand	14.2 N from casein....	3.42	2.88	....	....
7	Colby	14.2 N from casein....	....	....	....	lost
8	Peat	14.2 N from casein....	3.08	2.74	....	....

GROUP B.—LIMED SERIES.

1	Miami	14.2 N from $(NH_4)_2SO_4$	10.57	17.42	6.85	48.2
2	Sand	14.2 N from $(NH_4)_2SO_4$	2.84	3.03	trace	....
3	Colby	14.2 N from $(NH_4)_2SO_4$	6.26	11.90	5.64	39.7
4	Peat	14.2 N from $(NH_4)_2SO_4$	3.40	2.69	....	....
5	Miami	14.2 N from casein....	10.57	15.87	5.30	37.3
6	Sand	14.2 N from casein....	2.84	3.07	.23	....
7	Colby	14.2 N from casein....	6.26	12.50	6.24	43.9
8	Peat	14.2 N from casein....	3.40	2.51	....	....

When the Sparta sand or peat was used as inoculum no increase in nitrates occurred. At the end of 5 weeks no gain in nitrates was noted in the ammonium sulphate series, except where inoculated with Miami soil. Here there was a slight oxidation of the ammonium salt, about 8 per cent of the added nitrogen. In the casein series, Miami inoculation, the formation of nitrates proceeded much more rapidly; more than three times as much nitrate nitrogen was found. Unfortunately the results of the Colby inoculation were lost.

From the data of Group B, it will be seen that carbonate of lime under the conditions of this experiment has greatly stimulated the nitrification of ammonium sulphate. The percentage of nitrate nitrogen was greater in acid soil inoculated with Miami than in acid soil inoculated with Colby. When inoculated with acid sand or peat, no nitrification occurred. Where

casein nitrogen was used and the base soil inoculated with Miami, calcium carbonate did not prove so beneficial. In Group A, no lime, 24.6 per cent of the casein nitrogen was recovered as nitrate; in Group B, limed, 37.3 per cent. The greatest increase in nitrates in the casein series was noted in the soil inoculated with Colby; the smallest, in the soil inoculated with Sparta sand.

TABLE V.

RATE OF NITRIFICATION IN STERILIZED PEAT.

November 25—January 30.

GROUP A.—UNLIMED SERIES.

Culture	Inoculum	Treatment per 25 gm. of soil	Nitrate nitrogen per 25 gm. dry soil			
			Amount in control	After 9 weeks	Increase	Nitrogen recovered as nitrate
		Mg.	Mg.	Mg.	Mg.	%
1	Miami	28.4 N from $(NH_4)_2SO_4$	trace	3.73	3.73	13.1
2	Sand	28.4 N from $(NH_4)_2SO_4$	trace	trace	....	....
3	Colby	28.4 N from $(NH_4)_2SO_4$	trace	trace	....	....
4	Peat	28.4 N from $(NH_4)_2SO_4$	trace	trace	....	....
5	Miami	28.4 N from casein....	trace	9.61	9.61	33.8
6	Sand	28.4 N from casein....	trace	trace	....	....
7	Colby	28.4 N from casein....	trace	7.81	7.81	27.5
8	Peat	28.4 N from casein....	trace	trace	....	....

GROUP B.—LIMED SERIES.

November 25—January 8.

Culture	Inoculum	Treatment per 25 gm.of soil	Nitrate nitrogen per 25 gm. dry soil			
			Amount in control	After 6 weeks	Increase	Nitrogen recovered as nitrate
		Mg.	Mg.	Mg.	Mg.	%
1	Miami	28.4 N from $(NH_4)_2SO_4$	4.16	14.5	10.4	36.7
2	Sand	28.4 N from $(NH_4)_2SO_4$	trace	trace	....	....
3	Colby	28.4 N from $(NH_4)_2SO_4$	2.00	13.88	11.88	41.8
4	Peat	28.4 N from $(NH_4)_2SO_4$	trace	trace	....	....
5	Miami	28.4 N from casein....	4.16	13.9	9.74	34.4
6	Sand	28.4 N from casein....	trace	trace	....	....
7	Colby	28.4 N from casein....	2.00	12.5	10.5	36.9
8	Peat	28.4 N from casein....	trace	trace	....	....

Peat.—In Table V are shown the results of a study of nitrate formation in peat. Apparently sterilized acid peat is fairly well suited for the growth of the nitrifying organisms. As regards the source of nitrogen, the organic compound casein nitrifies much more rapidly than ammonium sulphate. There was a slight oxidation of ammonium sulphate in the peat inoculated with Miami soil. In the presence of calcium carbonate, inorganic nitrogen from ammonium sulphate nitrifies somewhat more rapidly than organic nitrogen from casein.

The inoculated but unlimed peat nitrifies slowly. This is shown from the figures of Table V.

A review of the tests in sterilized soils shows that nitrification takes place to a marked degree in certain acid soils. The form of the nitrogen to be oxidized plays an important part. Organic nitrogen from casein will nitrify much more rapidly in acid soil than inorganic nitrogen from ammonium sulphate. In the presence of calcium carbonate the nitrifying flora from Miami neutral soil or Colby acid soil will oxidize a large percentage of the nitrogen from inorganic or organic compounds. In the absence of calcium carbonate, the oxidation of ammonium sulphate is greatest where Miami soil is used as an inoculum. At this time no satisfactory explanation can be offered to account for the peculiar action of the organisms from Colby soil. It is hoped that the results of experiments now in progress will reveal some of the important agencies that affect nitrification.

TABLE VI.

RATE OF NITRIFICATION IN MIAMI SOIL.

January 16—January 19.

GROUP A.—FORMATION OF AMMONIA.

Culture	Treatment per 100 gm. of soil	Ammonia nitrogen per 100 gm. dry soil		
		Average	Increase due to treatment	Nitrogen in casein recovered as ammonia
	Mg.	Mg.	Mg.	%
1	None	3.38	.....	.....
2	100 $CaCO_3$	3.50	.12	.....
3	30 N from casein	12.84	9.46	31.53
4	30 N from casein plus 100 $CaCO_3$	13.44	10.06	33.53

GROUP B.—FORMATION OF NITRATES.

November 24—December 28.

Culture	Treatment per 100 gm. of soil	Nitrate nitrogen per 100 gm. dry soil			
		At beg.	At end	Increase	Nitrogen recovered as nitrate
	Mg.	Mg.	Mg.	Mg.	%
1	None	2.5	3.15	.65	.....
2	100 $CaCO_3$	2.5	3.15	.65	.....
3	14.2 N from $(NH_4)_2SO_4$	2.5	7.25	4.75	28.8
4	14.2 N from $(NH_4)_2SO_4$ plus 100$CaCO_3$	2.5	9.70	7.20	52.1
5	14.2 N from casein	2.5	8.33	5.83	36.4
6	14.2 N from casein plus 100 $CaCO_3$	2.5	8.95	5.80	36.2

AMMONIFICATION AND NITRIFICATION IN NON-ACID AND ACID SOILS.

Here 200-gm. portions of the various soils, except where otherwise indicated, were weighed into glass tumblers. After treatment, these were covered loosely with petri dishes and allowed to stand for varying intervals. Since it was arranged to use organic nitrogen, it is important to know the rate at which the nitrogenous substance is converted into ammonia. A comparison of nitrate formation in soil to which ammonium sulphate and casein have been added is not fair unless the rate of ammonia formation is known. However, it is very unlikely that nitrification is retarded because of the rate of ammonia production. As a rule, the formation of ammonia from casein is very rapid.

TABLE VII.

RATE OF NITRIFICATION IN SPARTA SAND.

January 17—February 20.

GROUP A.—FORMATION OF AMMONIA.

Culture	Treatment per 100 gm. of soil	Ammonia nitrogen per 100 gm. dry soil		
		Average	Increase due to treatment	Nitrogen in casein recovered as ammonia
	Mg.	Mg.	Mg.	%
1	None	1.33	.....	.....
2	522 $CaCO_3$	2.59	1.26	.....
3	30 N from casein	14.65	13.32	44.10
4	30 N from casein plus 522 $CaCO_3$	17.08	14.49	48.03

GROUP B.—FORMATION OF NITRATES.

Culture	Treatment per 100 gm. of soil	Nitrate nitrogen per 100 gm. dry soil			
		At beg.	At end	Increase	Nitrogen recovered as nitrate
	Mg.	Mg.	Mg.	Mg.	%
1	None	.5	.78	.28	....
2	522 $CaCO_3$	.5	3.12	2.62	....
3	14.2 N from $(NH_4)_2SO_4$	.5	.80	.30	....
4	14.2 N from $(NH_4)_2SO_4$ plus 522 $CaCO_3$	.5	5.95	5.45	20.0
5	14.2 N from casein	.5	3.00	2.50	12.8
6	14.2 N from casein plus 522 $CaCO_3$	.5	5.60	5.10	17.5

Miami soil.—A series of experiments were made to test ammonia and nitrate formation from casein. The amount of nitrogen used in ammonification tests, 30 mg. per 100 gm. of soil, was over twice as great as that used in the nitrification tests.

The results of this study are combined in Table VI, which is subdivided into Group A, ammonia formation, and Group B, nitrate formation. From the evidence presented in the table, two facts stand out very prominently. First, the nitrogen of casein ammonifies and nitrifies al-

most as fast with as without calcium carbonate. It is true that calcium carbonate in this test showed a slight increase in ammonia production, while the results of a second experiment gave no increase for liming. Second, the process of nitrification from ammonium sulphate is favored by calcium carbonate. A repetition of the experiment gave similar results. It seems probable that the benefit derived from the use of calcium carbonate is due to its basic properties. The carbonate reacts to neutralize the acids formed in the oxidation of ammonium sulphate.

Sparta sand.—The results of the test with Sparta sand are given in Table VII.

Here again, it is apparent that calcium carbonate at the rate of 0.522 per cent, enough to neutralize the active soil acidity, has very little effect on ammonification of casein. As might be expected, calcium carbonate exerts a very decided influence on the formation of nitrates from inorganic nitrogen. Ammonium sulphate in the absence of calcium carbonate did not nitrify, while casein without calcium carbonate gave a gain in nitrates of almost 13 per cent of the total nitrogen in casein. In the presence of calcium carbonate both substances nitrified, the ammonium sulphate more rapidly than the casein. Why nitrification in acid sand should take place under the conditions of this experiment and not in the previous tests (Table III), cannot be explained unless it is assumed that heat has in some way rendered the soil unfit for these nitrifiers. From the evidence, it seems that nitrification tests as performed in solution and in sterilized soil do not always furnish conclusive proof of the presence or absence of the nitrifying bacteria.

Colby silt loam.—The study of nitrification in Colby soil was carried out more in detail than the previous experiments. Since one of the main objects of this work is to note the relation between calcium carbonate and bacterial activity, it was arranged to give special consideration to the quantity necessary for optimum activity of the microörganisms. For this reason, the applications of calcium carbonate were made in three amounts. The first represents half enough to neutralize acidity; the second enough to neutralize acidity completely, and the third double enough to neutralize acidity according to the Truog method. A comparison of the effect of calcium carbonate on ammonification and nitrification is shown in Table VIII. In agreement with earlier tests, it will be seen that calcium carbonate has very little effect on the production of ammonia. The addition of calcium carbonate with ammonium sulphate caused a decided increase in nitrification. It is evident from the data of the table that the maximum nitrification after 3 weeks is reached with the largest amount of calcium carbonate.

In the casein series calcium carbonate produced the opposite effect. The formation of nitrates from casein in this soil seems to bear an in-

verse proportion to the amount of calcium carbonate applied. In the absence of calcium carbonate, casein nitrified almost three times as fast as ammonium sulphate.

TABLE VIII.

RATE OF NITRIFICATION IN COLBY SILT LOAM.

January 18—January 21.

GROUP A.—FORMATION OF AMMONIA.

Culture	Treatment per 100 gm. of soil	Ammonia nitrogen per 100 gm. dry soil		
		Average	Increase due to treatment	Nitrogen in casein recovered as ammonia
	Mg.	Mg.	Mg.	%
1	None	4.38	.....	.....
2	510 $CaCO_3$	4.34	.....	.....
3	1021 $CaCO_3$	3.68	.....	.....
4	2042 $CaCO_3$	4.10	.....	.....
5	30 N from casein	14.45	11.08	33.6
6	30 N from casein plus 510 $CaCO_3$	16.22	11.88	39.6
7	30 N from casein plus 1021 $CaCO_3$	15.86	12.18	40.6
8	30 N from casein plus 2042 $CaCO_3$	16.02	11.92	39.7

November 24—December 16.

GROUP B.—FORMATION OF NITRATES.

Culture	Treatment per 100 gm. of soil	Nitrate nitrogen per 100 gm. dry soil			
		At beg.	At end	Increase	Nitrogen recovered as nitrate
	Mg.	Mg.	Mg.	Mg.	%
1	None	3.57	4.13	.56	....
2	510 $CaCO_3$	3.57	5.30	1.73	....
3	1021 $CaCO_3$	3.57	6.70	3.13	....
4	2042 $CaCO_3$	3.57	7.10	3.53	....
5	14.2 N from $(NH_4)_2SO_4$	3.57	6.50	2.93	16.6
6	14.2 N from $(NH_4)_2SO_4$ plus 510 $CaCO_3$	3.57	11.90	8.33	46.0
7	14.2 N from $(NH_4)_2SO_4$ plus 1021 $CaCO_3$	3.57	13.80	10.23	49.6
8	14.2 N from $(NH_4)_2SO_4$ plus 2042 $CaCO_3$	3.57	14.80	11.28	54.5
9	14.2 N from casein	3.57	10.80	7.23	46.9
10	14.2 N from casein plus 510 $CaCO_3$	3.57	11.90	8.33	46.1
11	14.2 N from casein plus 1021 $CaCO_3$	3.57	12.50	8.93	40.6
12	14.2 N from casein plus 2042 $CaCO_3$	3.57	8.30	5.03	10.5

Peat.—The conditions that obtain in peat are so very different from those of the heavier soils that it is difficult to get any comparable data. The calcium carbonate in Group A was added in an amount great enough to neutralize half of the soil acidity. In Group B enough was applied to neutralize all of the acidity.

A glance at Table IX shows that the addition of calcium carbonate greatly benefited ammonia and nitrate formation. Here again, casein nitrified without the addition of any basic substance. In the presence of

calcium carbonate an increase in nitrates from casein is noted, but this is not nearly so great as the gain in nitrates from ammonium sulphate plus calcium carbonate.

TABLE IX.

RATE OF NITRIFICATION IN PEAT (WYEVILLE).

January 19—January 22.

GROUP A.—FORMATION OF AMMONIA.

Culture	Treatment per 100 gm. of soil	Ammonia nitrogen per 100 gm. dry soil		
		Average	Increase due to treatment	Nitrogen in casein recovered as ammonia
	Mg.	Mg.	Mg.	%
1	None	3.03	.....	.....
2	855 $CaCO_3$	5.69	2.66	.....
3	30 N from casein	8.88	5.85	19.5
4	30 N from casein plus 855 $CaCO_3$	21.65	15.96	53.2

May 8—June 6.

GROUP B.—FORMATION OF NITRATES.

Culture	Treatment per 100 gm. of soil	Nitrate nitrogen per 100 gm. dry soil			
		At beg.	At end	Increase	Nitrogen recovered as nitrate
	Mg.	Mg.	Mg.	Mg.	%
1	None	0.52	2.46	1.94	....
2	21.7 N from $(NH_4)_2SO_4$	0.52	1.86	1.34	....
3	21.7 N from $(NH_4)_2SO_4$ plus 1710 $CaCO_3$	0.52	16.74	16.22	65.7
4	20.3 N from casein	0.52	6.79	6.27	21.3
5	20.3 N from casein plus 1710 $CaCO_3$	0.52	12.52	12.02	49.6

THE EFFECT OF CALCIUM CARBONATE ON AMMONIFICATION AND NITRIFICATION IN COLBY SILT LOAM.

The retarding effect of calcium carbonate on nitrification in certain soils has been reported by Beckwith and his associates. They found the decrease in nitrate nitrogen to be greater with blood-meal than with ammonium sulphate; moreover, that the nitrifying bacteria are influenced by the reaction of the soil; acidity inhibits and likewise "Too much calcium carbonate does not seem best for their growth" (2).

A study of the influence of calcium carbonate on nitrification was undertaken. Two experiments were carried out, using casein and gelatin. The tests were similar in every respect except in the amount of organic nitrogen employed. Periodic determinations were made of the ammonia and nitrates. In one case 15 mg. were used, in the other 30 mg. The procedure was the same as in the foregoing experiments. It seems that the lower nitrate content in the presence of calcium carbonate may be due to one or two factors. First, calcium carbonate may be directly harmful

to the growth of the nitrifying organisms in this soil type; second, calcium carbonate may retard nitrate accumulation by stimulating the growth of those organisms that feed on nitrates.

If calcium carbonate in the amount employed in the preceding experiments is injurious to the nitrifying bacteria, it seems that the decrease in nitrates should be most noticeable soon after it is applied. In order to test this, a series of experiments were planned in which it was arranged to study nitrification at varying intervals.

Fifteen milligrams of nitrogen.—In all, five determinations were made. The results of this experiment are presented in Table X.

TABLE X.

RATE OF NITRIFICATION IN COLBY SILT LOAM.

June 19—August 13.

Culture	Treatment per 100 gm. of soil	Time after	Nitrate nitrogen per 100 gm. of dry soil				
			Control	Casein		Gelatin	
			Average	Average	Nitrogen recovered as nitrate	Average	Nitrogen recovered as nitrate
	Mg.	Days	Mg.	Mg.	%	Mg.	%
1	None	Beg.	2.04	2.02	....	1.96	....
2	1021 $CaCO_3$...	Beg.	2.12	2.12	....	2.07	....
3	None	8	3.79	5.91	14.1	5.98	14.6
4	1021 $CaCO_3$...	8	4.99	9.89	32.6	11.64	44.3
5	None	14	4.25	7.49	21.6	7.10	19.0
6	1021 $CaCO_3$...	14	5.32	13.14	52.1	12.97	51.0
7	None	28	5.47	12.99	50.1	11.61	40.9
8	1021 $CaCO_3$...	28	14.55	20.36	38.7	21.09	43.6
9	None	42	7.01	15.69	58.0	13.87	45.7
10	1021 $CaCO_3$...	42	16.35	24.21	52.4	22.89	43.6
11	None	56	7.82	16.61	58.6	16.87	60.3
12	1021 $CaCO_3$...	56	18.18	25.78	50.6	24.54	42.4

No study of ammonia formation was made in the first experiment. From the data of the tests, it will be seen that calcium carbonate greatly stimulated the formation of nitrates from casein and gelatin. This was most marked in analyses after 8 to 14 days. For example, as compared with control, the percentage gain on the eighth day for casein is 231, for gelatin 303. Six days later the relative increase in the calcium carbonate series was not so great. From the fourth week until the end of the experiment, the percentage of nitrogen recovered as nitrate from casein in the calcium carbonate series was below that of the unlimed series.

The beneficial effect of calcium carbonate on the accumulation of nitrates in the soil to which no nitrogen was added was noticeable after 8 days. From then until the end of the experiment in the limed soils, there was a gradual increase in amount of nitrate nitrogen. After 8 weeks the calcium carbonate controls contained more than double as much nitrogen as the blanks.

Thirty milligrams of nitrogen.—As a check on the results given in Table X, a similar experiment was planned in which double the amount of organic nitrogen was used. If the data of Table X are correct, calcium carbonate should cause an increase at first in nitrification and later a decrease. In view of the larger amount of organic nitrogen added in this test, it is probable that the period of decrease will not be noted until much later.

TABLE XI.

RATE OF NITRIFICATION IN COLBY SILT LOAM.

April 1—April 9.

GROUP A.—FORMATION OF AMMONIA.

Culture	Treatment per 100 gm. of soil	Time after	Ammonia nitrogen per 100 gm. dry soil from casein		Ammonia nitrogen per 100 gm. dry soil from gelatin	
			Average	Nitrogen in casein rec'd as ammonia	Average	Nitrogen in gelatin rec'd as ammonia
	Mg.	Days	Mg.	%	Mg.	%
1	None	2	11.37	37.9	8.59	28.6
2	1021 $CaCO_3$.....	2	14.35	47.8	10.92	36.4
3	None	4	16.82	56.0	11.60	38.6
4	1021 $CaCO_3$.....	4	21.84	72.8	14.49	48.3
5	None	6	19.35	64.5	14.68	48.9
6	1021 $CaCO_3$.....	6	24.22	80.7	18.34	61.6
7	None	8	20.68	68.9	13.00	43.3
8	1021 $CaCO_3$.....	8	24.29	80.9	15.39	51.3

April 1—June 26.

GROUP B.—FORMATION OF NITRATES.

Culture	Treatment per 100 gm. of soil	Time after	Nitrate nitrogen per 100 gm. of dry soil				
			Control	Casein		Gelatin	
			Average	Average	Nitrogen recovered as nitrate	Average	Nitrogen recovered as nitrate
	Mg.	Days	Mg.	Mg.	%	Mg.	%
1	None	Beg.	2.10	1.93	.....	1.93	.....
2	1021 $CaCO_3$....	Beg.	2.20	1.92	.....	1.92	.....
3	None	8	2.77	4.37	5.33	3.93	3.86
4	1021 $CaCO_3$....	8	3.80	8.75	16.50	8.75	16.50
5	None	14	5.96	7.19	4.10	7.95	4.63
6	1021 $CaCO_3$....	14	11.03	21.50	34.90	19.80	29.20
7	None	28	7.18	18.01	36.40	17.36	33.60
8	1021 $CaCO_3$....	28	11.34	25.67	47.76	23.33	39.96
9	None	42	8.95	22.43	44.90	20.47	38.40
10	1021 $CaCO_3$....	42	12.43	32.59	67.20	27.57	50.40
11	None	56	8.00	21.96	46.50	24.18	53.90
12	1021 $CaCO_3$....	56	15.00	31.65	55.50	30.79	52.90

A glance at the data in this table confirms the foregoing statement. The maximum nitrate content is not reached until after the eighth week. Ammonification takes place very rapidly in this soil type, reaching a

maximum at the end of the sixth day. Calcium carbonate increased the rate of ammonia formation. The subsequent decrease in the gelatin series on the eighth day may be due to an oxidation of the ammonia to nitrate.

In order to see if the reduction in nitrates is accompanied by a loss in nitrogen, an attempt was next made to measure the ammonia evolved from the limed and the unlimed soil. The results indicated that only a very slight amount of nitrogen escapes as free ammonia. Apparently calcium carbonate and casein in the amounts employed did not cause an appreciable loss of nitrogen as free ammonia.

Since the results of ammonia determinations failed to explain the loss of nitrate nitrogen, a new series of tests was made. In this case, total nitrogen analyses were made at the beginning and at the end of the experiment. The results of analyses showed that the carbonate of lime produced no loss of nitrogen. In this soil type the injury from calcium carbonate is not a result of any detrimental effect of calcium carbonate on nitrifying bacteria. Calcium carbonate without casein or gelatin always benefited nitrification. Moreover, no injury from calcium carbonate was noted until about 50 per cent of the nitrogen was converted into nitrates. It seems that the decrease in nitrate nitrogen from the use of calcium carbonate and organic nitrogen is due to a conversion of nitrate nitrogen into an organic form and not to any injurious effect on the nitrifying organisms. Since the conditions are favorable for bacterial development, it seems probable that an increase in nitrate-assimilating organisms may cause a decrease in nitrate content of the soil.

According to Miller (7), carbonate of lime causes a slight increase in number of soil bacteria. The gain is not noticeable at first. However, after 42 days he found double as many bacteria in the treated as in the untreated soil. Somewhat similar data were obtained with Colby silt loam soil. In this case, 41 days after treatment the limed series contained almost double as many bacteria. Later counts showed even a greater gain.

In a new test it was arranged to measure nitrate formation and number of bacteria at varying intervals. The experiment was set up September 16 and ended December 22. According to Arnd, carbonate of lime benefits the denitrifying flora (1). The nature of the agent that reduces the nitrate content of soil is indicated from the data in Table XII.

From the data it will be seen that at first calcium carbonate greatly benefits the rate of nitrate formation from gelatin. Later the reverse is true, there is a decrease in nitrate content in the calcium carbonate series. The results of plate counts offer an explanation for this phenomenon. Wherever the soils were treated with calcium carbonate and organic nitrogen, the number of bacteria increased enormously. The gain in total number of bacteria in this case far exceeded the gain with carbonate of

lime alone. Apparently the great number of organisms in these soils results in a reduction of nitrate nitrogen. As regards the effect of different amounts of organic nitrogen, there is apparently no difference between 15 and 30 mg. of gelatin. From the results obtained with calcium nitrate in the presence of calcium carbonate, it appears that in the absence of added organic matter, the reduction of nitrates in Colby silt loam soil does not take place so rapidly.

Plate I shows the relative number of colonies on Heyden agar plates at the time of the third count, 41 days after treatment. The same amount of soil was used in each petri dish. A glance at the colonies indicates very

TABLE XII.

EFFECT OF VARIOUS SUBSTANCES ON THE RATE OF NITRIFICATION IN COLBY SILT LOAM.

September 16—December 22.

GROUP A.—NITRATE FORMATION.

Treatment per 100 gm. of soil	Time in	Nitrate nitrogen in 100 gm. of soil								
		Control Average	Gelatin 30 mg.		Gelatin 15 mg.		Calcium nitrate 15 mg. and gelatin 15 mg.		Calcium nitrate 30 mg.	
			Average	Nitrified	Average	Nitrified	Average	Nitrified	Average	Nitrified
Mg.	Days	Mg.	Mg.	%	Mg.	%	Mg.	%	Mg.	%
None	7	6.6	9.1	8.4	8.9	15.2	21.3	—2.0	36.51	99.7
2040 $CaCO_3$....	7	8.7	13.0	14.3	13.9	34.6	26.3	17.8	37.7	96.8
None	21	7.9	21.1	43.8	16.5	57.3	28.7	38.2	37.6	99.0
2040 $CaCO_3$....	21	13.7	30.9	57.4	20.5	45.1	38.2	63.0	42.0	94.3
None	41	10.0	35.2	84.0	22.7	84.1	39.1	93.7	42.4	107.8
2040 $CaCO_3$....	41	19.8	43.8	80.0	30.2	69.3	48.5	91.3	51.0	104.0
None	69	10.4	35.7	84.3	25.3	98.6	37.0	77.3	40.0	98.7
2040 $CaCO_3$....	69	20.4	43.1	75.6	33.6	87.3	48.1	84.0	48.5	93.7
None	77	11.4	34.6	77.3	24.2	85.3	37.3	72.6	40.9	98.3
2040 $CaCO_3$....	77	23.7	45.0	71.0	34.0	68.7	47.6	59.3	50.0	87.7

GROUP B.—NUMBER OF BACTERIA.

Treatment per 100 gm. of soil	Time in	Bacteria per gram of soil[1]			
		Control	Gelatin 30 mg.	Gelatin 15 mg.	Calcium nitrate 15 mg. and gelatin 15 mg.
Mg.	Days				
None	7	4,187	10,670	6,078	5,673
2040 $CaCO_3$.....	7	4,862	20,666	14,047	6,213
None	21	9,177	25,769	9,297	34,997
2040 $CaCO_3$.....	21	15,184	32,489	29,484	10,151
None	41	7,158	8,104	7,564	13,101
2040 $CaCO_3$.....	41	13,507	66,587	27,688	41,600
None	69	3,512	5,132	4,457	5,673
2040 $CaCO_3$.....	69	14,182	31,875	33,091	38,494
None	77	810	675	945	810
2040 $CaCO_3$.....	77	10,940	24,312	21,070	14,587

[1] Thousands omitted.

clearly the effect of gelatin and calcium carbonate on the multiplication of bacteria.

A comparison of the number of bacteria and the amount of nitrate nitrogen in limed soils shows that to a certain degree these two factors are reciprocal. The greater the number of bacteria the lower the nitrates. Since the increase in number of bacteria in certain of these soils results in greater assimilation of nitrates, it seems that this same fact should be noted in nutrient solutions containing nitrate nitrogen. Accordingly 100-c.c. portions of Giltay's solution in 150-c.c. Erlenmeyer flasks were inoculated with equivalent amounts of soil taken from the cultures of the previous experiment. The samples were drawn at the end of the 69-day period. After inoculation, the culture solutions were incubated for 36 hours at 28°C. Nitrate determinations, as well as plate counts, are given in Table XIII.

TABLE XIII.

EFFECT OF LIME CARBONATE ON NUMBER OF BACTERIA AND NITRATE REDUCTION.

No.	Treatment of the inocula	Mg. of nitrate nitrogen per 100 c.c. of solution			Nitrogen rec'd as nitrates	Bacteria added per gm. of soil[1]
		Begin'g	End	Loss		
	Mg.	Mg.	Mg.	Mg.	%	
1	None	12.4	10.00	2.40	80.0	3,512
2	2040 $CaCO_3$	12.4	8.02	4.38	64.0	14,182
3	30 gelatin	12.4	10.20	2.20	81.0	5,133
4	15 gelatin	12.4	9.70	2.70	77.6	4,457
5	30 gelatin plus 2040 $CaCO_3$	12.4	3.06	9.34	24.4	31,875
6	15 gelatin plus 2040 $CaCO_3$	12.4	2.20	10.20	17.2	33,091
7	15 $Ca(NO_3)_2$ nitrogen plus 15 gelatin	12.4	9.20	2.20	73.6	5,673
8	15 $Ca(NO_3)_2$ nitrogen plus 15 gelatin plus 2040 $CaCO_3$	12.4	3.20	8.40	25.6	38,494

[1] Thousands omitted.

The results of these experiments are very interesting. The soils with the highest number of bacteria reduced the nitrates of Giltay's solution most rapidly. A glance at the figures of the last two horizontal columns shows that the number of bacteria and the percentage of nitrate recovered, are inversely proportional. The data furnish additional proof that the organisms in the treated soils take up nitrate nitrogen in their bodies. The loss of nitrates in the presence of calcium carbonate and organic nitrogen may be accounted for in this way.

Accumulation of Nitrates in Various Soils.

It has been shown repeatedly that if soil is protected from leaching, a part of the nitrogenous substances will be converted into ammonia and

later into nitrates. Under field conditions ammonia rarely occurs in large quantities. It is nitrified almost immediately: therefore the accumulation of nitrates in uncropped soil will depend to a great degree on the rate of ammonification (8). In this work with the various soil types only the amount of nitrate nitrogen was determined. No attempt was made to measure ammonification. In order to secure maximum accumulation of nitrates, the experiment was allowed to run for a long period of time.

Accumulation of nitrates.—Fresh samples of soil were collected, carefully mixed and filled into 2-gallon jars. The jars were kept in the greenhouse at a temperature of about 28°C. and watered at regular intervals with distilled water. All determinations were made from duplicate jars. The average results are presented in Table XIV.

TABLE XIV.

ACCUMULATION OF NITRATES IN VARIOUS SOILS.
June 6, 1914—June 6, 1915.

Culture	Soil	Treatment per 100 gm. of soil	Nitrate nitrogen per 100 gm. of dry soil							
			At beg.	After 4 wks.	After 8 wks.	After 12 wks.	After 16 wks.	After 24 wks.	After 32 wks.	After 52 wks.
		Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.	Mg.
1	Miami	None	1.58	8.33	14.7	13.8	14.0	16.8	21.60	22.80
2	Sand	None	0.14	1.66	2.0	1.5	1.6	....	3.40	3.60
3	Sand	177 $CaCO_3$.....	0.14	1.50	2.4	1.1	2.0	3.7	[1]4.63	6.19
4	Colby	None	0.42	4.35	11.0	....	12.2	12.8	.....	25.60
5	Colby	161 $CaCO_3$.....	0.42	2.86	11.5	12.5	12.4	14.4	[1]19.84	31.67
6	Peat	None	0.52	8.82	9.7	13.6	....	20.8	20.00	25.14
7	Peat	1710 $CaCO_3$.....	0.52	9.90	12.7	17.9	17.7	36.2	[1]35.00	73.44

[1] Denotes second application of calcium carbonate.

The results of Table XIV show the very great accumulation of nitrates which is noticeable in the absence of any added base. In the neutral soil there was a gradual rise in nitrate nitrogen during the entire experiment. The total accumulation of nitrate nitrogen in this soil is somewhat greater than that reported in an earlier publication (5, 6). With the exception of Wyeville peat, the accumulation of nitrates was slower in the acid soils than in the neutral Miami soil. As shown by the figures of the calcium carbonate series, the nitrate continued to accumulate up to the thirty-second week. It has been noted repeatedly that ammonia and nitrate accumulation in uncropped soil will proceed up to a certain amount, when it ceases (3, p. 178; 9). Because of the small gains in nitrate nitrogen towards the latter part of the experiment, calcium carbonate was added again to the soils of jars 3, 5 and 7. The amount in this case was the same as that used in the beginning. If the decreased rate of nitrification is a result of too much acid, then a second application should in all probability cause an increase in nitrates. The figures in the last column furnish proof for this statement. In order

to bring out this point more clearly, acidity determinations were made. The accumulation of nitrates should increase soil acidity, that is, provided the supply of basic substances in soil is low. It is of interest to note the general course of nitrification in acid soils.

Effect on reaction.—Table XV gives the results of acidity tests. (Veitch method.)

TABLE XV.

LIME REQUIREMENT OF SOILS USED FOR NITRATE ACCUMULATION.

Soil	Treatment per 100 gm. of soil	Calcium carbonate required to neutralize 100 gm. of dry soil	
	Mg.	At beginning Mg.	At end Mg.
Miami	None	Alkaline	Alkaline
Sand	1. None	177.8	147.2
	2. (a) Initial 177 $CaCO_3$		
	(b) After 32 weeks 177 $CaCO_3$	.....	31.6
Colby	1. None	161.7	264.4
	2. (a) Initial 161 $CaCO_3$		
	(b) After 32 weeks 161 $CaCO_3$	.....	39.2
Peat	1. None	1710.0	1172.0
	2. (a) Initial 1710 $CaCO_3$		
	(b) After 32 weeks 1710 $CaCO_3$	.....	Alkaline

It is evident that the initial amount of calcium carbonate added to the various acid soils was insufficient to counteract the acidity. The Miami silt loam, which was well supplied with basic elements at the beginning, still showed a neutral reaction, indicating that ample bases were present to care for any accumulation of acidity.

Sand under ordinary conditions is deficient in carbonates. After a period of 52 weeks this soil showed a lower calcium carbonate requirement than at the beginning of the experiment. This may be explained partly by the fact that distilled water was used throughout the entire period.

The Colby silt loam presented a different situation. Here, after a period of 52 weeks, a marked increase in acidity is evident from the data in Table XV. This increased acidity is most probably caused in part by the large accumulation of nitrate nitrogen. The initial calcium carbonate added was not sufficient to keep the soil in good nitrifiable condition. This is shown in the increased production of nitrate nitrogen after the second application, ranging from 19.84 mg. before to 31.67 mg. after treatment.

The peat soil showed a lower calcium carbonate requirement after the period than before. Here, the nitrate nitrogen content was high. No explantion for this result is offered at this time unless it is assumed that the bacteria have destroyed some of the organic acids. After the second application of calcium carbonate, a marked increase in nitrate nitrogen resulted.

In this connection, it is well to know the percentage of the total nitrogen that was converted into nitrates. The Miami silt loam contained 202.8 mg. of total nitrogen and showed an increase of 21.22 mg. of nitrate nitrogen, or 10.4 per cent of the total nitrogen.

The sand untreated showed an increase of only 3.46 mg. of nitrate nitrogen with 106.8 mg. of total nitrogen or 3.2 per cent. The treated sand showed an increase of 6.05 mg. nitrate nitrogen, or 5.6 per cent. Treatment of sand with calcium carbonate caused an increased production of nitrate nitrogen. The increase in the treated soil was 2.4 per cent greater than in the untreated.

Colby untreated showed an increase of 25.18 mg. of nitrate nitrogen with a total of 281.2 mg. or 8.95 per cent of the total nitrogen. The treated soil showed an increase of 31.25 mg. or 11.1 per cent of the total nitrogen. Calcium carbonate resulted in an increased oxidation of 2.15 per cent of organic nitrogen.

Peat untreated showed an increase of 24.62 mg. of nitrate nitrogen with a total of 1229.6 mg., or 2 per cent. The treated soil showed an increase of 72.92 mg., or 5.9 per cent. Treatment of peat with calcium carbonate stimulated nitrate production. The increase amounted to 3.9 per cent.

It is apparent from the foregoing figures that a large amount of the total nitrogen found in acid soils is converted into nitrate nitrogen. This amount is increased materially where acid soils are treated with calcium carbonate.

SUMMARY.

The formation of ammonia from casein takes place so rapidly in acid soils that for several weeks after the nitrogenous substance is added, the production of nitrates is not limited by lack of ammonia. The formation of nitrates in acid sand and acid Wyeville peat takes place very slowly. In acid Colby silt loam or the neutral Miami silt loam, nitrification takes place much more rapidly. The feeble nitrifying power of the sand and peat, as shown by inoculating these soils with an active culture of the nitrifying bacteria, is largely due to the condition of the soil. Apparently the nitrifying flora of Colby silt loam when transferred to a neutral soil is as active in the formation of nitrates as the flora from Miami silt loam.

In the case of the acid soils, it seems that the nature of the compound to be nitrified plays an important part. For example, in acid soils organic nitrogen nitrifies much more rapidly than nitrogen from ammonium sulphate. In non-acid soils the reverse is true, ammonium sulphate nitrifies more rapidly. This is true regardless of the source of the nitrifying bacteria. From the data, it seems that acid soils do not possess a strain of nitrifying bacteria especially resistant to soil acidity.

In the presence of organic nitrogenous substances as casein and gelatin, calcium carbonate did not permanently increase the accumulation of nitrates. For a short interval, one or two weeks, calcium carbonate stimulates nitrate formation; later the reverse is true and there is a decided decrease in the treated series. Apparently the reduction of nitrates is largely due to bacteria. It has been found that in the treated soil there is an enormous multiplication of the nitrate assimilating bacteria.

When stored under conditions that prevent leaching, all of the soils showed a gain in nitrate nitrogen. It seems that in Colby silt loam nitrification increases soil acidity and thus it becomes necessary to add a basic substance in order to keep the process going.

Before the results of the laboratory tests can be applied to field practice, it will be necessary to study nitrate formation on field plots.

Considering the data as a whole, it seems that under laboratory conditions, the beneficial effect of calcium carbonate on plant growth must be accounted for by some processes other than the direct effect on nitrification. The beneficial effect of calcium carbonate on nitrification takes place before higher plants begin to draw heavily on the nitrogen of nitrates. Moreover, the period of rapid accumulation from liming may result in a loss of nitrogen from leaching of the nitrates. The results of field tests should give an answer to these questions.

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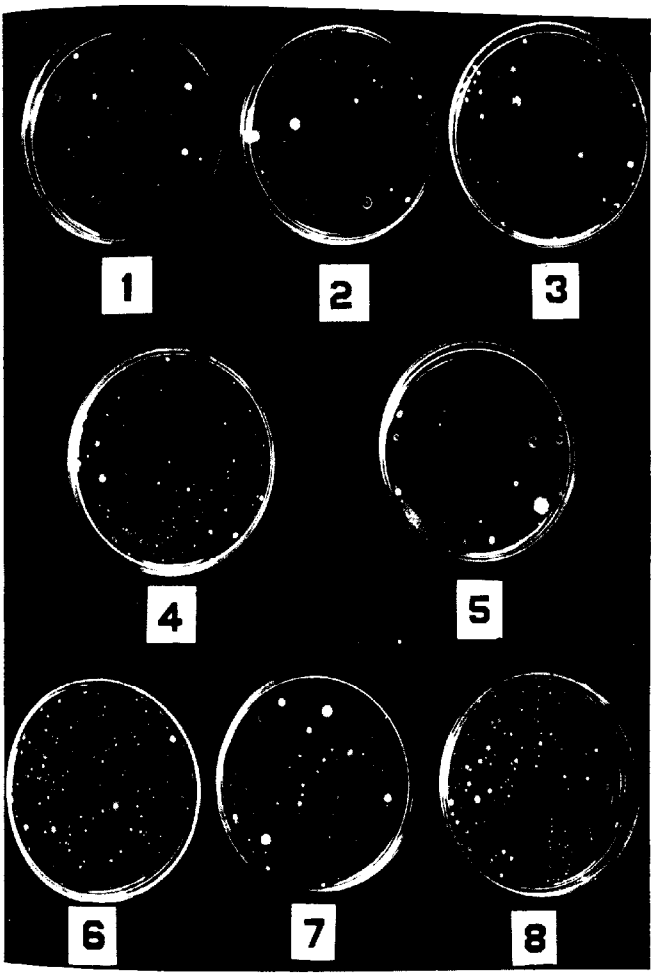
PLATE I.

Colonies of bacteria on Heyden agar plates in nitrification studies of Colby Silt Loam.

Fig. 1.—Control.
Fig. 2.—Control plus $CaCO_3$
Fig. 3.—Small amount of Gelatin.
Fig. 4.—Small amount of Gelatin plus $CaCO_3$
Fig. 5.—Large amount of Gelatin.
Fig. 6.—Large amount of Gelatin plus $CaCO_3$
Fig. 7.—Gelatin plus $Ca(NO_3)_2$
Fig. 8.—Gelatin plus $Ca(NO_3)_2$ plus $CaCO_3$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STUDIES IN SULFOFICATION.[1]

BY

P. E. BROWN, *Chief in Soil Chemistry and Bacteriology,* AND H. W. JOHNSON, *Assistant in Soil Bacteriology, Iowa Agricultural Experiment Station.*

I.

Recent experiments (1) have shown that sulfofication or sulfur-oxidation is an important process occurring in field soils. Plants have been found to require considerable amounts of sulfates for their best growth and inasmuch as sulfur is not present in soils in that form but as unassimilable organic and inorganic compounds, it is apparent that the ability of a soil to produce sulfates from these unavailable substances will determine very largely the sulfur-feeding of the crops grown. In other words, the total sulfur content, alone, of a soil will not show the sulfur available for plant growth. The sulfofying or sulfate-producing power of the soil must also be ascertained.

The investigations mentioned besides demonstrating the fact that all soils possess a definite sulfofying power which is determinable in the laboratory, threw considerable light upon the conditions governing the process. Thus it was found that additions of green manure and barnyard manure increased the sulfofying power of the soil, and in general, that the treatment which the soil had undergone influenced considerably its ability to produce sulfates. Furthermore, the optimum moisture content of the soil for the occurrence of the process was found to be 50 per cent of the amount necessary for complete saturation, and the oxidation of sulfur was found to occur to the greatest extent in a mixture of 50 per cent soil and 50 per cent sand, showing the importance of aeration. Again the addition of carbohydrates to the soil was shown to depress sulfofication, the greater the amount added, the greater the depression, and the depression also varied in the inverse ratio to the solubility of the carbohydrates.

A definite laboratory method was devised for determining the sulfofying power of soils and this consisted in the addition of 0.1 gm. of Na_2S or free sulfur, preferably the latter, to 100-gm. quantities of fresh soil, adjustment of the moisture content to the optimum for the soil, and incubation for from 5 to 10 days. The sulfates were then determined by shaking the soil with water for 7 hours in the shaking machine, filtering, precipitating the sulfates with barium chloride, and estimating in the sulfur photometer.

[1] Received for publication March 10, 1916.

Studies on the sulfur content of Iowa soils confirm the observations of Hart and Peterson (2) in Wisconsin and Shedd (3) in Kentucky, that on the average much less sulfur than phosphorus was present in the various large soil areas. Some of the sulfur removed from the soil by crops may, of course, be returned by the use of manure, but the amount of manure produced on a livestock farm is quite insufficient, as a general rule, to keep up the sulfur content of the soil, and unless manure is purchased or large amounts of commercial feeds are used, commercial sulfur-containing fertilizers must be applied to maintain the soils permanently fertile.

This does not mean that applications of sulfur fertilizers would prove profitable on Iowa soils at the *present time*, but it does mean that unless different methods of soil treatment are employed than those at present in use, at *some future time*, sulfur will be lacking. In other words for *permanent soil fertility*, the sulfur supply for crops must be considered.

It is known at the present time that the amount of phosphorus present in Iowa soils is low, and in many cases this element may be the limiting factor of growth. Acid phosphate and rock phosphate are the two materials which are available commercially for supplying phophorus. The former supplies sulfur as well as phosphorus and the question arises whether it may have any superior value because of the sulfur added. The relative merits of the two phosphorus compounds are not yet definitely known, and it is possible that the sulfur content, and also the effect on sulfofication, should be considered in selecting the material which should be recommended for remedying deficiencies of phosphorus in soils.

The relative effects of the materials mentioned on sulfofication, on ammonification, and on crop yields should also be ascertained. If sulfofication and ammonification run parallel, it will be evident that methods of treatment which are of value because of a stimulation of nitrate production will also lead to greater sulfate production. If crop yields and sulfofication are similarly affected, it may be that the effects of the materials are largely due to the sulfur factor.

Therefore, the following experiment was planned to throw some light upon the problem of the relative effects of gypsum, acid phosphate, rock phosphate, alone and with gypsum, and mono-calcium phosphate on sulfofication, on ammonification and on the yields of oats in pots in the greenhouse.

The Plan of the Experiment.

The soil used in this work was a Carrington Loam, high in organic matter and having a basic reaction. When analyzed, it was found to contain 911 pounds of phosphorous and 2487 pounds of sulfur per acre of two million pounds of surface soil. This sulfur content was very high, much higher than that of any of the samples of Iowa soils whose analyses were given in the bulletin already referred to.

The results secured were undoubtedly modified considerably because of the presence of so much sulfur in the soil used. The use of gypsum for instance could hardly be expected to show any effect and all of the applications would probably exert much more influence on sulfofication in a soil lower in sulfur.

This soil was evidently somewhat abnormal for Iowa conditions, and hence the results should not be interpreted as of more than technical and special interest.

Twenty-four 4-gallon pots were filled with the soil described which was in an air-dried condition, 27.6 pounds being placed in each pot.

The special treatments were as follows:

Pots.	*Treatment.*
1- 2.	Check.
3- 4.	24.7 lbs. calcium sulfate per acre.
5- 6.	70.5 lbs. mono-calcium phosphate per acre.
7- 8.	300 lbs. acid phosphate per acre.
9-10.	1000 lbs. rock phosphate per acre.
11-12.	1000 lbs. rock phosphate + 24.7 lbs. $CaSO_4$ per acre.
13-14.	Check.
15-16.	24.7 lbs. $CaSO_4$ per acre.
17-18.	70.5 lbs. $CaH_4(PO_4)_2$ per acre.
19-20.	300 lbs. acid phosphate per acre.
21-22.	1000 lbs. rock phosphate per acre.
23-24.	1000 lbs. rock phosphate + 24.7 lbs. $CaSO_4$ per acre.

Pots 13 to 24 were seeded to oats and the remainder were kept bare for bacteriological tests.

The applications were based on actual field conditions, the 300 pounds of acid phosphate and 1000 pounds of rock phosphate forming the basis of the additions. The acid phosphate was analyzed for phosphorus and sulfur and showed a content of 5.2 per cent of phosphorus and 1.994 per cent of sulfur. The applications of calcium sulfate and mono-calcium phosphate made to the soil were calculated, so that the same amounts of sulfur and phosphorus, respectively, as were applied in the acid phosphate should be added.

The rock phosphate contained 5.85 per cent of phosphorus and hence considerably more phosphorus was applied than in the case of the acid phosphate, but the amounts of both materials used were those commonly employed on the farm, and hence a fair comparison was provided. The variation in available phosphorus, of course, accounts for the difference in the amounts applied.

The optimum moisture content of the soil was determined and after the pots were filled, distilled water was applied to bring all the soils up to

that content. The pots were then weighed and during the continuance of the experiment the moisture content was maintained by additions of distilled water to weight.

The oats were harvested just prior to maturity, the green and dry weights secured and the nitrogen content determined.

Samples were drawn for bacteriological tests every two weeks, the sulfates present were determined, the moisture content ascertained and tests for sulfofying power by the free-sulfur-fresh-soil method, previously described, and for ammonification by the casein-fresh-soil method and the dried-blood-fresh-soil method were carried out.

The usual precautions were observed in sampling to secure uncontaminated samples. The sulfate determinations were made by shaking the soil with water for 7 hours in the shaking machines as usual. The ammonia determinations were made by the magnesium-oxide method.

The experiment was begun on January 11th, and the samples were drawn on January 26th, February 9th, February 23rd, March 9th and March 23rd.

The Sulfates Present at Sampling.

The amounts of sulfates present in the soils at the various samplings are given in Table I, and the average contents under the different treatments are calculated.

On examining the table it appears that there was little variation in sulfate content at the different samplings. The amounts added were very small and evidently the method used in the subsequent determinations was not sufficiently accurate to show them.

There are some indications in all the samplings, except the last, of a depression in sulfate content in the treated soils, but the differences were too small to be distinctive. The later sulfofying tests showed increases in sulfofying power, due to treatment, and hence it would hardly be reasonable to assume any depression in sulfate content in this case. The variations in results should, therefore, probably be regarded as due to the method of determination and as indicating the absence of any effect of the materials added, rather than as distinctive differences.

It is apparent, however, that the variations in sulfate content from one sampling to the next were very slight, much smaller than is usually the case with nitrates. There are such variations, however, that the conclusion seems justified that sulfate production and assimilation vary in much the same way that nitrate production and assimilation vary. That is, there may be an accumulation up to a certain point which is followed by increased assimilation and hence a decrease in the amount present. In the field, of course, there are losses in sulfates by leaching and assimilation by plants, just as in the case of nitrates, but in these experiments there was no leaching and no plants grown and hence the differences

were due to variations in production and assimilation by bacteria. There are evidently certain sulfate-assimilating bacteria which may become very active in the presence of abundance of sulfates and whose activity declines as the amounts of sulfates present are used up.

It will be left for future experiments to learn more of these sulfate-assimilating bacteria. Their activities may be a source of removal of sulfates from the use of crops, but it is more probable that they would be regarded as a means of preserving sulfates in the soil and preventing the loss by leaching. Sulfates which are used by the assimilating bacteria would later become available again for plant growth, and hence at times of too large sulfate production for the needs of crops. These bacteria would prove of much value in preventing losses by leaching.

TABLE I.

SULFUR AS SULFATES PRESENT AT TIME OF SAMPLING.

Pot No.	Treatment	Lab No.	1st Samples		2nd Samples		3rd Samples		4th Samples		5th Samples	
			Mg. S. as SO_4	Av. for Treatment	Mg. S. as SO_4	Av. for Treatment	Mg. S. as SO_4	Av. for Treatment	Mg. S. as SO_4	Av. for Treatment	Mg. S. as SO_4	Av. for Treatment
1	Check	1	4.98		6.22		5.95		5.20		5.36	
		2	5.07		7.27		5.81		5.71		4.48	
2	Check	3	4.88		7.27		6.87		6.53		6.68	
		4	5.17	5.02	7.27	7.00	7.28	6.50	6.29	5.93	6.52	5.75
3	$CaSO_4$	5	3.91		5.81		6.36		5.88		5.84	
		6	4.75		5.61		6.36		6.12		6.92	
4	$CaSO_4$	7	3.87		5.40		5.61		5.88		5.36	
		8	5.17	4.43	5.71	5.64	5.71	6.01	5.61	5.87	5.52	5.91
5	$CaH_4(PO_4)_2$	9	4.83		6.36		7.21		6.12		6.84	
		10	4.83		7.82		7.14		6.46		6.56	
6	$CaH_4(PO_4)_2$	11	4.54		5.61		5.11		4.93		5.68	
		12	5.17	4.84	5.34	6.28	5.71	6.44	4.59	5.52	5.68	6.19
7	Acid Phos.	13	4.70		5.27		6.87		5.24		6.28	
		14	5.37		5.44		6.02		5.78		6.60	
8	Acid Phos.	15	4.83		4.56		5.34		4.83		6.12	
		16	5.17	5.02	4.83	5.02	5.27	5.89	5.24	5.27	6.33	6.33
9	Rock Phos.	17	3.64		4.25		4.56		4.69		5.52	
		18	4.39		4.18		4.49		4.69		5.12	
10	Rock Phos.	19	3.51		4.25		4.39		4.96		5.36	
		20	4.70	4.01	4.35	4.26	4.56	4.49	4.76	4.77	5.52	5.38
11	R'k Ph. + $CaSO_4$	21	4.99		5.27		6.46		7.07		6.84	
		22	5.17		5.44		6.36		6.60		6.84	
12	R'k Ph. + $CaSO_4$	23	3.79		5.40		6.53		5.20		5.76	
		24	4.88	4.71	5.40	5.37	6.38	6.43	5.44	6.07	6.08	6.38

It is evident also from these results that sulfates do not accumulate in soils any more than nitrates do. They seem to be subject to much the same influences as nitrates, and this fact suggests that sulfate production and also sulfate assimilation are very closely related, respectively, to nitrate production and nitrate assimilation, and that the influence of certain known factors on the nitrogen processes may be the same on the sulfur processes.

THE SULFOFICATION TESTS.

The samples drawn on the dates given previously, were tested for their sulfofying power according to the method described,—the free-sulfur-fresh-soil method.

The results secured at the various samplings are given in Tables II, III, IV, V and VI and the average results for the different treatments appear in Table VII.

TABLE II.

PER CENT OF ADDED SULFUR, SULFOFIED, 1ST SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as SO_4	Av. Mg. S. as SO_4	Mg. S. as SO_4 in soil after incubation	Av. Mg. S. as SO_4	Mg. S. as SO_4 from S. added	% S. Sulfofied for each treatment
1	Check	1	lost		7.75			
		2	lost		7.36	7.55		
2	Check	3	38.76		8.46			
		4	36.72	36.74	8.23	8.33	28.40	28.40
3	$CaSO_4$	5	43.69		7.47			
		6	42.84	43.26	7.47	7.47	35.79	
4	$CaSO_4$	7	43.69		7.34			
		8	45.90	44.78	7.34	7.34	37.44	36.60
5	$CaH_4(PO_4)_2$	9	40.46		7.92			
		10	42.50	41.48	8.09	8.00	33.48	
6	$CaH_4(PO_4)_2$	11	48.62		7.27			
		12	41.48	45.05	7.41	7.34	37.71	35.60
7	Acid Phos.	13	42.50		7.75			
		14	41.99	42.24	7.75	7.75	34.49	
8	Acid Phos.	15	38.76		5.88			
		16	36.38	37.57	5.57	5.72	31.85	33.20
9	Rock Phos.	17	43.69		5.71			
		18	41.16	42.41	5.71	5.71	36.70	
10	Rock Phos.	19	32.64		5.27			
		20	32.64	32.64	5.44	5.35	29.29	33.00
11	R'k Ph. + $CaSO_4$	21	27.06		7.27			
		22	36.38	36.72	7.58	7.42	29.30	
12	R'k Ph. + $CaSO_4$	23	36.04		6.22			
		24	34.68	35.36	6.35	6.28	29.08	29.20

On examining the results given in the complete tables, it is found that the duplicate determinations agreed very closely, indicating that the method employed in the estimation of the sulfates was quite satisfactory.

The results from the duplicate pots were not always in perfect agreement, but that is ever the case in greenhouse experiments. Differences in the location of duplicate pots with reference to the glass, seem to exert an important influence on the bacteriological results as well as on the crop yields secured in the greenhouse. Of course, there is the danger of accidental contamination in soils under such abnormal conditions as pertain in the greenhouse, as indicated by the growth of algae, which is frequently observed, the occurrence of molds and possibly also of proto-

zoans. But, in general, the differences in the heat and light relations may account for many of the variations which are encountered.

The results secured in this work from the duplicate pots were as uniform as is usually the case and whatever the causes of the variations may be, it was impossible to ascertain them, and hence the average results must be considered as fairly accurate.

TABLE III.

PER CENT OF ADDED SULFUR, SULFOFIED, 2ND SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as SO_4	Av. Mg. S. as SO_4	Mg. S. as SO_4 in soil after incubation	Av. Mg. S. as SO_4	Mg. S. as SO_4 from S. added	% S. Sulfofied for each treatment
1	Check	1	32.30		7.99			
		2	31.11	31.70	8.19	8.09	23.16	
2	Check	3	28.07		7.99			
		4	29.75	28.91	8.74	8.36	20.25	21.72
3	$CaSO_4$	5	31.11		7.28			
		6	31.11	31.11	7.21	7.24	23.87	
4	$CaSO_4$	7	35.19		6.97			
		8	36.89	36.04	6.87	6.92	29.12	26.50
5	$CaH_4(PO_4)_2$	9	35.19		8.02			
		10	35.53	35.36	8.40	8.21	27.15	
6	$CaH_4(PO_4)_2$	11	35.19		5.75			
		12	36.38	35.78	5.75	5.75	30.03	28.60
7	Acid Phos.	13	28.07		7.28			
		14	30.60	29.33	7.07	7.17	22.16	
8	Acid Phos.	15	28.90		6.29			
		16	26.69	27.79	5.88	6.08	21.71	21.90
9	Rock Phos.	17	30.09		5.24			
		18	31.11	30.60	5.20	5.22	25.38	
10	Rock Phos.	19	31.79		5.34			
		20	29.24	30.01	5.05	5.19	24.82	25.10
11	R'k Ph. + $CaSO_4$	21	27.20		6.45			
		22	34.85	31.02	6.53	6.49	24.52	
12	R'k Ph. + $CaSO_4$	23	27.71		6.73			
		24	31.79	29.75	6.29	6.50	23.25	23.90

Each table gives the amounts of sulfates present in the soils after incubation, and upon examining these results and comparing them with the amounts of sulfates which the soils contained at sampling, given in Table I, it appears that the incubation of the soils for 10 days brought about only very slight changes in the sulfate content of the soils. It is evident, therefore, that the amount of sulfates present in soils does not change to any great extent in short periods of time. In other words in the absence of leaching and of assimilation by crops there seems to be somewhat of an equilibrium established between sulfate-production and sulfate-assimilation. At any rate, the sulfate content of soils under these conditions changes so slowly that tests made within short intervals of time do not seem to show any large differences.

Under field conditions it is quite probable that the differences would be much greater and would appear in a much shorter space of time. In short, it seems extremely doubtful if an equilibrium such as was found here would be established under field conditions in the presence of the disturbing factors of leaching and assimilation by crops. Unless special treatments were followed it would be reasonable to expect that the sulfate content of soils would gradually decline, and such is actually the case in the field. As the total sulfur content becomes less the production of sulfates becomes slower, as has been shown in the sulfofication studies already referred to. Hence under field conditions instead of an equilibrium in sulfates, a gradual decline is found unless special treatments are followed.

TABLE IV.

PER CENT OF ADDED SULFUR, SULFOFIED, 3RD SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as SO_4	Av. Mg. S. as SO_4	Mg. S. as SO_4 in soil after incubation	Av. Mg. S. as SO_4	Mg. S. as SO_4 from S. added	% S. Sulfofied treatment for each
1	Check	1	33.66		6.02			
		2	33.32	33.49	5.88	5.95	27.54	
2	Check	3	34.85		6.60			
		4	34.85	34.85	6.60	6.60	28.25	27.90
3	$CaSO_4$	5	39.10		7.28			
		6	39.61	39.35	6.97	7.12	32.23	
4	$CaSO_4$	7	43.69		5.71			
		8	35.70	39.65	5.88	5.79	33.86	33.00
5	$CaH_4(PO_4)_2$	9	34.34		7.44			
		10	35.36	34.85	7.28	7.36	27.49	
6	$CaH_4(PO_4)_2$	11	31.79		5.17			
		12	33.66	32.72	5.34	5.25	27.47	27.50
7	Acid Phos.	13	32.30		5.20			
		14	31.96	32.13	4.42	4.81	27.32	
8	Acid Phos.	15	34.34		4.69			
		16	33.66	34.00	5.13	4.91	29.09	28.20
9	Rock Phos.	17	37.23		4.79			
		18	35.36	36.29	4.69	4.74	31.55	
10	Rock Phos.	19	41.99		5.00			
		20	38.76	40.37	4.83	4.91	35.46	33.50
11	R'k Ph. + $CaSO_4$	21	39.95		5.85			
		22	41.99	40.92	6.19	6.02	34.90	
12	R'k Ph. + $CaSO_4$	23	32.30		5.95			
		24	34.00	30.15	5.95	5.95	27.20	31.00

Upon subtracting the sulfate content of the soils after incubation from the total amount of sulfates found in the tests, the remainder is calculated as per cent of sulfur sulfofied, and these are the figures which show the sulfofying power of the soils.

Turning to Table VII which gives the average percentages of sulfur sulfofied, some interesting facts become evident.

In the first place it is found that the calcium sulfate, even in the small applications made, increased to a marked degree the sulfofying power of the soil. This marked increase occurred at every date of sampling, and bears out the results secured in the earlier experiments already referred to, according to which calcium sulfate in various amounts increased the sulfofying power of the soil used to a large extent, the influence being in direct proportion to the size of the application. Of course, if the amount of sulfate applied were increased beyond a certain point it is probable that no further increase in sulfofication would occur and an actual depression might take place. The interesting feature of the present results is that very small amounts of gypsum, such as may be added to soils in another fertilizing material (acid phosphate), may exert a pronounced influence upon the ability of the soil to produce sulfates.

TABLE V.

PER CENT OF ADDED SULFUR, SULFOFIED, 4TH SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as SO_4	Av. Mg. S. as SO_4	Mg. S. as SO_4 in soil after incubation	Av. Mg. S. as SO_4	Mg. S. as SO_4 from S. added	% S. Sulfofied for each treatment
1	Check	1	34.6		6.92			
		2	34.2	34.4	6.92	6.92	27.5	
2	Check	3	33.6		5.48			
		4	34.4	34.0	5.76	5.62	28.4	27.9
3	$CaSO_4$	5	31.6		6.20			
		6	32.0	31.8	6.40	6.30	25.5	
4	$CaSO_4$	7	41.6		6.32			
		8	45.6	43.6	6.04	6.18	37.4	31.4
5	$CaH_4(PO_4)_2$	9	52.2		5.12			
		10	50.0	51.1	5.76	5.44	45.6	
6	$CaH_4(PO_4)_2$	11	43.2		5.08			
		12	44.2	43.7	4.96	5.02	38.7	42.1
7	Acid Phos.	13	48.8		6.88			
		14	46.6	47.7	6.84	6.86	40.8	
8	Acid Phos.	15	47.0		6.72			
		16	46.0	46.5	6.32	6.52	40.0	40.4
9	Rock Phos.	17	50.0		5.36			
		18	51.4	50.7	5.16	5.26	45.5	
10	Rock Phos.	19	47.0		5.40			
		20	43.8	45.4	5.40	5.40	40.0	42.7
11	R'k Ph. + $CaSO_4$	21	35.0		7.32			
		22	42.0	39.5	6.92	7.12	32.4	
12	R'k Ph. + $CaSO_4$	23	40.4		6.40			
		24	39.2	39.8	5.40	5.90	33.9	33.1

In other words, the effects of gypsum may be partly due to a stimulative action as has been supposed as well as to the addition of a plant food constituent. The stimulative action may be of considerable importance on soils which contain sufficient amounts of total sulfur but do not have a rapid enough sulfofying action. In other words if soils are found which

contain fairly large amounts of sulfur but on which crops are not supplied with sufficient sulfates for their best growth, applications of small amounts of gypsum might be sufficient to stimulate sulfofication to such an extent that the sulfur already present in the soil would be sulfofied rapidly enough to keep plants supplied with that element.

The mono-calcium phosphate gave considerable increases in sulfofication, and these were especially pronounced at the last two samplings. The increases were very similar to that brought about by the gypsum, varying somewhat from those results, as might be expected. It is apparent that this material exerted some stimulative action on sulfofication, and if it has any effect on crop growth, that effect might be considered to be due

TABLE VI.

PER CENT OF ADDED SULFUR, SULFOFIED, 5TH SAMPLES.

Pot No.	Treatment	Lab No.	Mg. S. as SO_4	Av. Mg. S. as SO_4	Mg. S. as SO_4 in soil after incubation	Av. Mg. S. as SO_4	Mg. S. as SO_4 from S. added	% S. Sulfofied for each treatment
1	Check	1	35.4		5.12			
		2	36.6	35.9	5.08	5.10	30.8	
2	Check	3	45.6		5.72			
		4	44.6	45.1	5.72	5.72	29.4	30.1
3	$CaSO_4$	5	36.6		5.88			
		6	47.6	48.1	6.16	6.02	41.1	
4	$CaSO_4$	7	45.6		5.40			
		8	44.6	45.1	5.40	5.40	39.7	40.4
5	$CaH_4(PO_4)_2$	9	45.6		5.88			
		10	43.2	44.4	5.88	5.88	38.6	
6	$CaH_4(PO_4)_2$	11	50.0		5.00			
		12	47.6	48.8	4.96	4.98	43.8	41.1
7	Acid Phos.	13	40.4		6.24			
		14	40.4	40.4	6.04	6.14	34.3	
8	Acid Phos.	15	48.8		5.68			
		16	45.6	47.2	5.56	5.62	41.6	37.9
9	Rock Phos.	17	47.0		5.52			
		18	46.6	46.8	5.16	5.34	41.5	
10	Rock Phos.	19	45.6		5.16			
		20	43.8	44.7	5.04	5.10	39.6	40.5
11	R'k Ph. + $CaSO_4$	21	45.6		7.68			
		22	47.6	46.6	6.44	7.06	39.5	
12	R'k Ph. + $CaSO_4$	23	46.0		6.04			
		24	49.4	47.7	6.04	6.04	41.7	40.6

in part to an increased production of sulfates and not entirely to the phosphorus supplied. The action of this material may be somewhat indicative of the effect of acid phosphate, assuming that the phosphorus in this latter material is in the mono-calcic form which it is in part at least.

The applications of acid phosphate increased the sulfofying power of the soil, but to a smaller extent in practically all cases than either the gypsum or the mono-calcium phosphate alone. It appears, therefore, that

on this soil the combination of the two substances was not as beneficial for sulfofication as either of them alone. Just why this should be the case is difficult to determine. It is probably, however, the result of more complicated bacterial changes brought about by the combined substances, although the other calcium phosphates present in the acid phosphate, such as the dicalcic and tricalcic phosphates may explain the different effects.

An interesting practical point is brought out here, however. The acid phosphate when applied to this soil had a stimulative action on sulfofication, and hence its influence on crop yields, if it exerts any effect whatever, may not be due entirely to the phosphorus which it supplies to the crops in available form or to the sulfate which is supplied, but in part to the increase in sulfate production from the soil. Previous suggestions regarding the value of acid phosphate because of effects on the sulfur feeding of plants are thus confirmed, and it seems reasonable to conclude that on soils deficient in both phosphorus and sulfur, acid phosphate would be a good material to use to supply both deficiencies, increasing the sulfates available for plants both by actual additions and by increased production in the soil.

TABLE VII.

PER CENT OF ADDED SULFUR, SULFOFIED FOR EACH TREATMENT AT EACH SAMPLING.

Treatment	Samples				
	1st	2nd	3rd	4th	5th
1. Checks	28.4	21.7	27.9	27.9	30.1
2. 24.7 lbs. $CaSO_4$	36.6	26.5	33.0	31.4	40.4
3. 70.5 lbs. $CaH_4(PO_4)_2$	35.6	28.6	27.5	42.1	41.1
4. 300 lbs. Acid Phosphate	33.2	21.9	28.2	40.4	37.9
5. 1000 lbs. Raw Rock Phosphate	33.0	25.1	33.5	42.7	40.5
6. 1000 lbs. Raw Rock Phosphate plus 24.7 lbs. $CaSO_4$	29.2	23.9	31.0	33.1	40.6

Raw rock phosphate applied at the rate of 1000 pounds to the acre, a normal farm application, increased the sulfofying power of the soil to a greater extent than did the acid phosphate also applied in the customary field amount. The increase was about the same as that exerted by the mono-calcium phosphate. Only one reason suggests itself in explanation of the greater influence of the rock phosphate over the acid phosphate—that it is due to the greater amount of phosphate used. Perhaps the sulfofying bacteria use phosphorus in their growth and the stimulative effect of phosphorus fertilizers on sulfofication is really due to a feeding of the sulfofiers. In such a case, which seems quite probable, the question arises as to in what form the phosphorus is required by the bacteria. Probably it must be in a soluble form when it would be expected that the acid phosphate would give greater increases than the rock phosphate.

It is interesting to consider this effect of rock phosphate on sulfofication from the practical standpoint. If raw rock phosphate will stimulate sulfate production to as large an extent as these results indicate, it may be that the material would be quite as valuable as a phosphorus *and sulfur* fertilizer such as acid phosphate, at least on soils not extremely low in sulfur. In other words, if rock phosphate will stimulate sulfate production from soils sufficiently to supply the needs of crops, it may be unnecessary to use a special sulfur fertilizer except in extreme cases, and the phosphorus fertilizer may be depended on for a dual purpose. Of course, this is assuming that the rock phospate gives as good effects from the phosphorus standpoint as the acid phosphate, a point which, as has been mentioned, is far from being settled at the present time.

When gypsum was applied with the rock phosphate, increases in sulfofication were noted, but these gains were smaller than those secured with the rock phosphate alone and smaller also than those given by the gypsum alone. The increases were about the same as those given by the acid phosphate. It is apparent again, therefore, that the single constituents gave more effect than the two together. In this case, also, just as with the acid phosphate, the cause for this smaller increase with the combined materials is not apparent and may be due to complicated bacterial changes where the two substances were combined. It is evident that on soils not very deficient in total sulfur, rock phosphate alone may prove just as beneficial as when applied with gypsum because of a greater production of available sulfates.

It must be emphasized again that these results apply to this particular soil only and not to soils in general. The soil used in this work was unusually high in sulfur, as has been pointed out, and hence the effects of sulfur fertilizers would be less pronounced than on soils poorer in sulfur. If there are such pronounced effects on the sulfofying power of this particular soil by small applications of the various fertilizing materials, a much greater effect might be expected from the same substances on a soil poorer in sulfur, or a more normal soil.

Therefore, the following conclusions from this work seem entirely justified, and while they apply specifically to this particular soil, they may be found to be of much more general application:

Applications of acid phosphate, of rock phosphate, of gypsum, of rock phosphate and gypsum, and of mono-calcium phosphate increased the sulfofying power of the soil to a considerable extent.

The rock phosphate, mono-calcium phosphate and gypsum gave the largest increases, larger than those given by the mixtures or by the acid phosphate.

Any of these materials, therefore, when applied to the soil in normal field amounts may be expected to increase sulfate production. Their ef-

fects on crops grown, if any, may be due partly at least to this influence on sulfur transformation. It is particularly interesting to note the greater effect of the rock phosphate than of the acid phosphate on sulfofication. On soils not strongly depleted in sulfur, therefore, but deficient in sulfofication, and also in need of phosphorous, it seems possible that the rock phosphate would prove as satisfactory as the more soluble acid phosphate. Crop yields must, of course, prove this point before it can be accepted definitely.

No reason can be assigned for the greater effects on sulfofication of the single constituents over the combinations. They were probably due to complicated bacterial processes which the latter engendered, and about which nothing is known as yet.

THE AMMONIFICATION EXPERIMENTS.

The samples drawn on the dates already mentioned were tested for their ammonifying power by the casein-fresh-soil method and the dried-blood-fresh-soil method. The former method was employed at the first

TABLE VIII.

AMMONIFICATION TESTS.

Pot No.	Treatment	Lab No.	Mg. N. as NH_3	Av. for Treatment	Mg. N. as NH_3	Av. for Treatment	Mg. N. as NH_3	Av. for Treatment	Mg. N. as NH_3	Av. for Treatment	Mg. N. as NH_3	Av. for Treatment
1	Check	1	86.08		82.49		247.2		268.2		212.4	
		2			83.45		235.4		247.5		229.9	
2	Check	3	86.46		81.53		277.2		288.7		212.1	
		4		86.27	82.49	82.49	282.6	261.1	280.7	271.3	210.9	216.3
3	$CaSO_4$	5	84.53		80.57		292.9		309.7		246.5	
		6			82.49		283.2		291.9		236.6	
4	$CaSO_4$	7	83.76		83.45		292.3		309.7		211.2	
		8		84.14	83.93	82.64	281.4	287.5	294.6	301.5	238.3	233.1
5	$CaH_4(PO_4)_2$	9	88.01		83.05		292.3		300.1		213.0	
		10			83.45		284.1		295.9		237.9	
6	$CaH_4(PO_4)_2$	11	89.17		83.93		278.9		276.9		234.6	
		12		88.59	83.45	83.47	271.4	281.7	280.2	288.3	251.5	234.2
7	Acid Phos.	13	91.87		85.84		306.5		301.2		249.3	
		14			83.45		284.4		289.6		210.3	
8	Acid Phos.	15	93.03		80.09		282.9		279.7		222.9	
		16		92.45	80.09	82.36	277.2	287.8	282.8	288.3	234.3	228.9
9	Rock Phos.	17	87.62		83.05		285.1		291.9		212.4	
		18			85.84		305.6		275.6		203.7	
10	Rock Phos.	19	86.85		82.01		269.0		280.7		211.4	
		20		87.38		83.22	283.4	285.8	276.9	281.3	214.3	210.4
11	R'k Ph. + $CaSO_4$	21	87.23		83.93		274.5		300.2		226.6	
		22			85.84		272.6		283.5		220.5	
12	R'k Ph. + $CaSO_4$	23	87.23		85.36		235.9		293.8		217.9	
		24		87.23	87.28	85.60	230.6	257.9	253.4	282.7	210.9	218.9

and second samplings, the incubation period being 3 and 5 days respectively, but the results were not satisfactory, and the remaining tests were made by the dried-blood method. All of the results secured are given in Table VIII.

The duplicates were much more satisfactory where the casein was used, but the effects of the treatments were not clearly pronounced; the difference in ammonifying power of the soil were too small in many cases to be conclusive. The dried-blood results were more definite, but the same difficulty which is usually met with was encountered with them, that is, the impossibility of securing entirely satisfactory duplicate determinations. However, the results given in the table show certain tendencies among the treatments and it will be worth while to call attention briefly to some points which appear more or less definitely.

The calcium sulfate had the greatest effect of any of the materials on ammonification, the mono-calcium phosphate and acid phosphate were about equal in their effect, but lower than that of the calcium sulfate or the raw rock phosphate, and rock phosphate with calcium sulfate had little influence. Practically all the differences between these averages are as great as between duplicates.

The stimulative action of all these materials on ammonification is indicated by the results secured, and there are some relations evident between the ammonification results and the sulfofication tests. Thus in both cases the calcium sulfate exerted the greatest stimulative action of any of the materials used. In the sulfofication results, however, the rock phosphate alone gave practically as large an effect as the gypsum, while in ammonification it had less influence.

Again, in the sulfofication tests the acid phosphate had less effect than either the calcium sulfate or mono-calcium phosphate, while in ammonification it showed less influence than the calcium sulfate, but practically the same as the mono-calcium phosphate. In both cases the mixture of rock phosphate and calcium sulfate gave small influence.

It is impossible to explain these divergences in results, some of which owing to the difficulties encountered in the methods are not as pronounced as they should be, and indeed it is doubtful if the present results should be regarded as conclusively showing any definite differences in effect on ammonification among the various substances used. Not a large enough number of determinations were made and the duplicate results were not in sufficiently satisfactory agreement.

The stimulative action of all the substances on ammonification was, however, indicated, just as was the case with sulfofication, and hence there must be some relationship between the two processes. Of course, the same groups of organisms are not involved in the different processes, but they may belong in the same class because of their requirements for growth, especially their food materials and the most favorable mechanical soil conditions.

The differences noted in the effect of phosphorus fertilizers may have been due to different effects of phosphorus as a food material on the two

groups of bacteria, but as has been pointed out these variations were not distinctive and it is probable that the food material requirements of the different groups are not very dissimilar.

The Crop Yields.

The oats were harvested just prior to maturity, and the green and dry weights secured. The crop was analyzed for nitrogen and the removal of nitrogen from the soil in the crop was calculated. All these results are given in Table IX.

TABLE IX.

THE CROP YIELDS.

Pot No.	Treatment	Green Weights Gm.	Average	Dry Weight Gm.	Average	% N. in Crop	Gm. N. in Crop	Average
1	Check	266.00		52.90		2.572	1.3605	
2	Check	262.40	264.2	49.70	51.30	2.423	1.1942	1.2773
3	$CaSO_4$	263.55		50.45		2.310	1.1654	
4	$CaSO_4$	243.90	253.7	49.00	49.72	2.346	1.1495	1.1574
5	$CaH_4(PO_4)_2$	239.60		48.00		2.677	1.2849	
6	$CaH_4(PO_4)_2$	271.35	255.4	50.45	49.22	2.201	1.1104	1.1976
7	Acid Phosphate	327.80		62.70		2.699	1.6923	
8	Acid Phosphate	319.90	323.9	63.00	62.85	2.561	1.6134	1.6528
9	Raw Rock Phosphate	300.00		55.00		2.751	1.5121	
10	Raw Rock Phosphate	323.70	311.9	56.50	55.75	2.652	1.4984	1.5052
11	Raw Rock Phos. plus $CaSO_4$	305.30		54.90		2.959	1.6244	
12	Raw Rock Phos. plus $CaSO_4$	277.40	291.3	51.70	53.30	2.553	1.3200	1.4722

On examining the table it is found that all the applications of phosphorous except the mono-calcium phosphate increased the crop yield. The acid phosphate gave the largest increase, much larger than that given by the raw rock. When the gypsum was applied with the rock phosphate, slightly lower yields were secured than when the rock phosphate alone was used. The difference, however, was slight and should not be considered as indicating any depression from the use of the gypsum.

The gypsum alone and the mono-calcium phosphate gave no effects. The actual average yields were slightly less than that of the check soils, but the differences in the duplicate pots were as great as those between the checks and the treated soils, and hence the results should merely indicate an absence of effect for the treatments.

It will be recalled that the soil used in this work was very low in phosphorus and hence a beneficial effect of the phosphorus fertilizers on crop yields might have been expected. It is evident from these results that when a soil is as low in phosphorus as this one was, applications of phosphorus fertilizers would prove of value. These results also indicate a superior value for the acid phosphate over the rock phosphate. No conclusions applicable to field conditions should be drawn from this single experiment, especially as it was conducted under greenhouse conditions.

The results may merely serve to indicate what *may* occur under field conditions *on this particular soil type*. No attempt has been made, therefore, to calculate the relative cost of applications and the value of the increases, which would be necessary in field tests, in order to arrive at some conclusions regarding the relative values of the applications.

Why the mono-calcium phosphate should not have brought about any increase in yield is not apparent from the results. A slight depression in the crop yield was actually observed, but it was not large enough to be distinctive, as the differences in the duplicate pots were wider than the differences between the check and treated pots, as has been noted. It appears merely, therefore, that the plants were unable to utilize the phosphorus from this compound as well as from acid phosphate. The sulfate present in the acid phosphate could not account for the greater effect of the latter material as the sulfate alone produced no effect on the crop. Possibly the acidity of the mono-calcium phosphate may explain the results, especially as this would have more effect in the absence of the calcium sulfate than where the two occur together in the acid phosphate.

The use of calcium sulfate on this soil was definitely shown to be of no value. This is as might be expected from the fact that the soil was so abnormally high in sulfur. There was evidently an abundance of sulfur present and in the presence of sufficient organic matter and lime, the process of sulfofication proceeded rapidly enough to keep the oats supplied with sulfates. Even in the presence of phosphorus, where a larger growth was secured, the sulfate had no additional effect, showing the absence of any need for sulfates on this soil.

On comparing the results of the sulfofication tests and ammonification tests with the crop yields, it is found that there were some agreements and some discrepancies in the effects of the various treatments. The gypsum exerted the greatest effect on sulfofication and likewise on ammonification, but had no influence on the crop grown. Mono-calcium phosphate likewise gave a considerable increase in sulfofying power and in ammonifying power, but had no effect on the yield of oats. Acid phosphate however increased sulfofication, ammonification and crop yield, the latter to the greatest extent of any material used, and the two former processes to as great an extent as the other substances applied. Rock phosphate increased the crop yield and the sulfofying power of the soil, but had no pronounced effect on ammonification. It is apparent, therefore, that the sulfofying power of a soil may be increased without a corresponding increase in crop yield occurring. As has been mentioned, conclusions should hardly be drawn from the ammonification results, but it seems that other factors might be of greater importance from the crop standpoint than from the standpoint of the transformation of soil nitrogen at least, in greenhouse soils.

In general, these crop yield results show that on this soil, rich in sulfur but poor in phosphorus, phosphate fertilizers produced a pronounced effect, while sulfates had no influence. The supply of sulfur and of nitrogen available for plant growth was evidently sufficient and phosphorus was the limiting factor of growth. Hence the influence of applications of materials merely increasing the supply of nitrates and sulfates was not apparent above the effect of the use of phosphorus.

CONCLUSIONS.

This experiment leads, therefore, to the following conclusions:

1. The sulfate content of the soil varied only slightly from one sampling to the next. There were no sudden or striking changes in the amount of sulfates present in the soil, kept fallow in the greenhouse.

2. The sulfate content of soils in the field is subject to the same influences as the nitrate content, but the effects are probably much less pronounced.

3. Calcium sulfate, mono-calcium phosphate, acid phosphate, rock phosphate and rock phosphate plus gypsum increased the sulfofying power of the soil. The sulfate alone and phosphates alone had greater effects than combinations of the two materials as in acid phosphate.

4. All the materials used increased the ammonifying power of the soil, but the differences between the effects of the various substances were not pronounced. The rock phosphate had less effect, however, than the other materials.

5. The sulfofication tests and ammonification tests did not always run parallel, although very similar effects of the materials used, on the two processes were noted.

6. The phosphorus fertilizers, except mono-calcium phosphate, increased the yield of oats, the acid phosphate to a greater extent than the rock phosphate. The sulfate had no effect on the crop yield. Such results were expected on this soil rich in sulfur but deficient in phosphorus. The lack of effect from the mono-calcium phosphate was probably due to the acidity, which was of more effect in the absence of the sulfate than when the two were together as in the acid phosphate.

7. The crop yields, sulfofication and ammonification results were not always parallel. In general it appeared that on this soil increases in sulfofication were not necessarily parallel with increases in yields. The ammonification results were not conclusive but indicate that materials supplying plant food constituents which are lacking in the soil may be of double value because of increases in the production of other plant food constituents in an available form.

II.

Series I.

The Proper Incubation Period for Tests of Sulfofication.

In previous tests of soils for their sulfofying power by the use of free sulfur, which was found to be the best material to use, the incubation period was 10 days. It seemed desirable to ascertain whether this period of incubation allowed the greatest differentiation between soils from different sources and under varying treatments. Shorter periods of incubation were eliminated, as less satisfactory in earlier experiments and hence the tests here were carried out at 7, 10, 12 and 14 day periods.

Five soils, very different as to texture and composition, and thus presumably varying widely in sulfofying power, were selected. Fresh soil was used, being weighed out in 100-gm. quantities in tumblers, 100 mg. of sulfur added to each, and the moisture content of each of the soils adjusted to the optimum for that particular soil. The sulfates produced at the end of the various incubation periods were determined in the usual way.

TABLE X.

SULFATES PRESENT AFTER DIFFERENT PERIODS OF INCUBATION.

No.	Soils	S, as SO_4 after 7 da.	Average	S, as SO_4 after 10 da.	Average	S, as SO_4 after 12 da.	Average	S, as SO_4 after 14 da.	Average
1	Heavy black	5.72		11.76		7.92		21.47	
	Woodland soil	5.12	5.42	6.72	9.24	11.00	9.46	20.54	21.00
2	Typical sand	8.20		9.00		9.12		14.00	
	River-bank in sod	6.68	7.44	8.56	8.78	9.52	9.32	14.40	14.20
3	Humus Plot 107	5.84		5.00		4.88		6.10	
	Check	8.40	7.12	4.00	4.50	5.16	5.02	6.23	6.16
4	Humus Plot 101	9.88		10.80		18.33		30.80	
	Continuous timothy....	10.28	10.08	11.44	11.12	18.67	18.50	30.60	30.70
5	Corn-field soil	9.40		6.04		7.20		10.00	
	River terrace	8.08	8.74	6.55	6.30	8.56	7.88	10.20	10.10

On examining the results given in Table X, it is apparent that considerably larger amounts of sulfates were produced from the sulfur added, with the longer incubation periods. None of the soils showed more than a trace of sulfates at the beginning of the experiment, so the entire amount found at the end of the incubation period may be considered as produced from the sulfur added.

At the end of the 7-day period, the differences between the various soils were much too small in several cases to be conclusive. After 10 days' incubation, the amounts of sulfates produced were somewhat larger and the ranking of the soils in sulfofying power had changed materially. The duplicate determinations also agreed much better. In 12 days, the differences in sulfofying power had become still more pronounced, but the ranking of the soils was the same.

Again after 14 days' incubation, the variations among the soils were larger, but the ranking of the soils was the same as after the 10 and 12-day periods.

These results indicate, therefore, that when soils are tested for their sulfofying power by the free-sulfur-fresh-soil method, the tests should be incubated for at least 10 days to secure the proper ranking of the soils, and much better results may be secured by incubating the samples for 12 or even 14 days.

The greatest differences between various soils may be obtained by incubating for the longer period, that is, for 14 days.

SERIES II.

THE EFFECT OF GYPSUM ON SULFOFICATION.

In the earlier experiments already referred to, gypsum was found to exert a stimulative effect on sulfofication, but the amounts used were rather small and further tests seemed desirable to ascertain whether large applications would show a greater effect or whether they would depress the activities of the sulfofying bacteria. This series was planned to test this point. The soil used was a Marshall silt loam from Lee County, Iowa. It was air-dried, sieved through a twenty-mesh sieve and weighed out as usual. Sulfur in the usual amount and the special quantities of calcium sulfate were then added and thoroughly stirred in. Ten cubic centimeters of a soil infusion, made by shaking 100 gm. of fresh soil in 200 c.c. of water for five minutes were added and sufficient sterile water supplied to bring the moisture content up to the optimum. The tests were then incubated for 10 days, after which the sulfates were estimated as usual.

Table XI gives the arrangement of the experiment, together with the results secured. On examining these results, it appears that the smallest amount of gypsum had practically no effect on the sulfofying power of the soil, while the larger amounts depressed the production of sulfates. The greatest depression occurred with the use of 0.30 gm. of the sulfate, and when 0.50 gm. was added the depression was less but it was still greater than that with the 0.10 gm. of the sulfate.

The previous experiments which have shown the stimulating effect of gypsum were carried out in greenhouse soils and much smaller amounts were used than was the case here, so that these results are not in any way opposed to the earlier ones. It was apparent in those results that applications of gypsum at a rate sometimes employed in field soils stimulated sulfofication and hence it is evident that the application of gypsum cannot be increased to any appreciable extent without bringing about a depression in sulfofying power.

There could be no practical value, therefore, in making heavy applications of gypsum from the standpoint of bringing about an increase in sulfofication. Of course, these results should not be accepted as conclusive for field practice because of the fact brought out in earlier work that gypsum is rather readily assimilated in the soil, hence there was probably some assimilation in these experiments and the results secured for the treated soils may have been too small. It was impossible to ascertain the extent of the assimilation and in making the calculations, the total amount of sulfate added was subtracted from the final figure.

TABLE XI.

THE EFFECT OF $CaSO_4$ ON SULFOFICATION.

No.	Treatment	Mg. S. as SO_4 after Incubation	Mg. S. as SO_4 added in $CaSO_4$	Mg.S.as SO_4 from free S.	Av. Per Cent S. Sulfofied
1	Nothing	21.0	0	21.00	
2	Nothing	21.6	0	21.60	21.30
3	0.05 gm. $CaSO_4$	28.4	9.35	19.05	
4	0.05 gm. $CaSO_4$	30.6	9.35	21.25	20.15
5	0.075 gm. $CaSO_4$	33.0	14.02	18.98	
6	0.075 gm. $CaSO_4$	32.0	14.02	17.98	18.48
7	0.10 gm. $CaSO_4$	37.6	18.69	18.91	
8	0.10 gm $CaSO_4$	36.4	18.69	17.71	18.31
9	0.30 gm. $CaSO_4$	62.8	56.07	6.73	
10	0.30 gm. $CaSO_4$	60.0	56.07	3.93	5.33
11	0.50 gm. $CaSO_4$	106.0	93.45	12.55	
12	0.50 gm. $CaSO_4$	110.0	93.45	16.55	14.55

It is safe to conclude, however, that the applications of gypsum which will give the most practically economic effect are those commonly employed in field practice.

SERIES III.

THE EFFECT OF CALCIUM CARBONATE ON SULFOFICATION.

If sulfofication is an important process occurring in field soils as seems to be the case, the effect of applications of calcium carbonate on its occurrence must be considered. Is it increased as are ammonification and nitrification, or it is decreased when the acidity of a soil is remedied by the use of limestone? This test was planned to throw some light on this point.

The same soil used in the preceding test was employed here. The soil was weighed out, the calcium carbonate in special amounts, and the sulfur added and stirred in, 10 c.c. of a fresh soil infusion applied, the moisture content adjusted to the optimum and the tests incubated for 10 days. The results of the sulfate determinations appear in Table XII. It appears clearly in this table that the use of calcium carbonate on an acid soil increased sulfofication. There was a considerable increase when the acid-

ity of the soil, the lime requirement of which was 1072 pounds per acre of 2,000,000 pounds of surface soil, was neutralized and with further additions of calcium carbonate still greater gains in sulfofication were found. The greatest gain, however, occurred with the use of 0.3 gm. per 100 gm. of soil, corresponding to 6000 pounds to the acre, and beyond that point the increases were somewhat less.

TABLE XII.

THE EFFECT OF $CaCO_3$ ON SULFOFICATION.

No.	Treatment	Mg. S. as SO_4 after Incubation	Average for Treatment
1	Nothing	21.0	
2	Nothing	21.9	21.4
3	Neutralized	27.2	
4	Neutralized	25.6	26.4
5	Neutralized plus 0.1 gm. $CaCO_3$	32.2	
6	Neutralized plus 0.1 gm. $CaCO_3$	32.8	32.5
7	Neutralized plus 0.3 gm. $CaCO_3$	40.6	
8	Neutralized plus 0.3 gm. $CaCO_3$	36.4	38.5
9	Neutralized plus 0.5 gm. $CaCO_3$	32.6	
10	Neutralized plus 0.5 gm. $CaCO_3$	33.4	33.0
11	Neutralized plus 1.0 gm. $CaCO_3$	35.0	
12	Neutralized plus 1.0 gm. $CaCO_3$	33.4	34.2
13	Neutralized plus 5.0 gm. $CaCO_3$	32.6	
14	Neutralized plus 5.0 gm. $CaCO_3$	30.4	31.5

If the applications of the carbonate had been increased still further, it is possible that the sulfofication would have decreased below that of the soil with its acidity just neutralized or even below that of the acid soil, but the amounts used here were not sufficiently large to bring about such a decrease. Inasmuch as the applications made in the field never exceed the amounts used here, there need be no apprehension of decreasing sulfofication by the use of ordinary amounts of calcium carbonate to remedy acid conditions in the soil.

On the other hand, it is evident from these results that calcium carbonate up to 6000 pounds per acre increased the sulfofying power of this soil. Larger amounts of the carbonate such as are rarely used in practice gave considerable increase in sulfofication, but these were somewhat less than those secured with the 3-ton amount.

SERIES IV.

THE EFFECT OF MAGNESIUM CARBONATE ON SULFOFICATION.

Having ascertained that calcium carbonate exerted a beneficial effect on sulfofication, it was deemed desirable to determine whether magnesium carbonate would have the same effect or not. This experiment was planned to throw some light on the question.

The soil used was the same as in the previous series. The arrangement of the test was the same as in the previous case except that no acid soil

was incubated and that magnesium carbonate was added in place of calcium carbonate. The check soils in this case were neutralized with calcium carbonate and all the other soils received additional amounts of magnesium carbonate.

On turning to Table XIII which gives the results of the tests, it is found that the smallest amount of magnesium carbonate increased slightly the sulfofying power of the soil, but the larger amounts gave gradually increasing depressions up to the largest amount employed here. It is evident that applications of magnesium carbonate in amounts greater than 2000 pounds per acre depressed the sulfofying power of this soil below that shown by the sample receiving no magnesium carbonate at all.

TABLE XIII.

THE EFFECT OF $MgCO_3$ ON SULFOFICATION.

No.	Treatment	Mg. S. as SO_4 after Incubation	Average for Treatment
1	Nothing	24.4	
2	Nothing	25.7	25.0
3	0.1 gm. $MgCO_3$	28.0	
4	0.1 gm. $MgCO_3$	31.4	29.7
5	0.3 gm. $MgCO_3$	26.5	
6	0.3 gm. $MgCO_3$	21.4	23.9
7	0.5 gm. $MgCO_3$	19.6	
8	0.5 gm. $MgCO_3$	17.1	18.3
9	1.0 gm. $MgCO_3$	17.0	
10	1.0 gm. $MgCO_3$	16.5	16.7
11	5.0 gm. $MgCO_3$	15.8	
12	5.0 gm. $MgCO_3$	15.1	15.4

Soil neutralized with $CaCO_3$.

Comparing these results with those secured with the calcium carbonate in the previous test, it is found that the use of magnesium carbonate at the rate of 2000 pounds per acre gave less effect on sulfofication than the use of the same amount of calcium carbonate, both additions being made to a neutralized soil.

While, however, the use of 3 tons of calcium carbonate per acre above that necessary to neutralize the acidity of the soil, increased the sulfofying power of the soil, the application of that amount of magnesium carbonate depressed sulfofication considerably.

It is apparent, therefore, that the application of magnesium carbonate to neutral soils should be made with care, and amounts greater than 2 tons per acre might depress the sulfofying power of the soil.

Evidently the sulfofying bacteria are much less sensitive to the presence of an abundance of calcium carbonate than to the presence of much magnesium carbonate. This is in accord with other bacteriological results dealing with the transformation of soil nitrogen, and it is in accord also with many crop results.

SERIES V.

THE EFFECT OF CALCIUM AND MAGNESIUM CARBONATES ON SULFOFICATION.

This test was planned to determine the effect of calcium and magnesium carbonates combined on sulfofication. The same soil and the same arrangement of the experiment was used here as in the two previous tests, except that both calcium and magnesium carbonates were applied. The amounts of these materials combined were the same as the amounts of the single substances used in the earlier series.

The results of the tests appear in Table XIV, and an examination of this table shows that the use of calcium and magnesium carbonates in amounts larger than 2000 pounds per acre of both together depressed the sulfofying power of this soil below that of the neutral soil. The check soils here represented the soil with its entire acidity neutralized with calcium carbonate. Increasing the application of calcium and magnesium carbonates together beyond 6000 pounds per acre decreased the sulfofying power of this soil, the depression increasing with increasing amounts applied.

TABLE XIV.

THE EFFECT OF $CaCO_3$ PLUS $MgCO_3$ ON SULFOFICATION.

No.	Treatment	Mg. S. as SO_4 after Incubation	Average for Treatment
1	Nothing	27.0	
2	Nothing	24.8	25.9
3	0.05 gm. $CaCO_3$ plus 0.05 gm. $MgCO_3$	28.0	
4	0.05 gm. $CaCO_3$ plus 0.05 gm. $MgCO_3$	20.0	29.0
5	0.15 gm. $CaCO_3$ plus 0.15 gm. $MgCO_3$	25.7	
6	0.15 gm. $CaCO_3$ plus 0.15 gm. $MgCO_3$	24.1	24.9
7	0.25 gm. $CaCO_3$ plus 0.25 gm. $MgCO_3$	18.2	
8	0.25 gm. $CaCO_3$ plus 0.25 gm. $MgCO_3$	21.4	19.8
9	0.50 gm. $CaCO_3$ plus 0.50 gm. $MgCO_3$	16.1	
10	0.50 gm. $CaCO_3$ plus 0.50 gm. $MgCO_3$	16.4	16.2
11	2.50 gm. $CaCO_3$ plus 2.50 gm. $MgCO_3$	19.8	
12	2.50 gm. $CaCO_3$ plus 2.50 gm. $MgCO_3$	22.3	21.0

It is apparent, therefore, that on this soil applications of calcium carbonate produced greater effects on sulfofication than the use of magnesium carbonate or combinations of the two carbonates. It is further evident the use of magnesium or dolomitic limestones on this soil, after its acidity has been neutralized with calcium carbonate may lead to a depression in sulfofying power, if the amounts used exceed 2000 pounds to the acre. On the other hand, non-magnesian limestones up to 6000 pounds per acre increased the sulfofying power of the soil, and in larger applications, produced smaller effects on sulfofication, but no actual depressions.

CONCLUSIONS.

These tests lead to the following conclusions:

1. In the use of the free-sulfur-fresh-soil method for testing the sulfofying power of soils, the incubation period should be 14 days at room temperature to give the most conclusive results. Ten day's incubation gave the relative sulfofying powers of soils quite accurately, but the differences were much more distinctive for the longer period.

2. Calcium sulfate in ordinary applications had no detrimental effect on sulfofication, but very large applications might decrease the rate of oxidation of sulfur.

3. Calcium carbonate in ordinary applications on acid soils, increased sulfofication considerably and even in excessive amounts affected sulfur oxidation favorably.

4. Magnesium carbonate in small amounts increased sulfofication, but in large amounts depressed it even below that in the same soil with its acidity unneutralized.

5. Magnesium carbonate and calcium carbonate in combination exerted a beneficial influence on sulfofication when used in small amounts. Larger applications, however, depressed the oxidation of sulfur. The effects of the combined material were less than those of the calcium carbonate alone.

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BACTERIAL NUMBERS IN SOILS, AT DIFFERENT DEPTHS, AND IN DIFFERENT SEASONS OF THE YEAR.[1]

By

SELMAN A. WAKSMAN,

Research Assistant, Agricultural Experiment Station, Rutgers College.

The latter half of the nineteenth century and the beginning of the twentieth witnessed the rapid development of the study of the biology of the soil. A great deal of work has been done along the line of types and physiological activities of the soil bacteria. This paper is limited to the investigation of the bacterial numbers in soils under different treatment, at different depths, and in different seasons of the year.

HISTORICAL.

The early investigators in the field of soil bacteriology were looking for pathogenic organisms. The importance of bacteria as factors in the fertility of the soil has been revealed as a result of these investigations. Birsh-Hirschfield, having studied a few Dresden soils in 1874, came to the conclusion that wet soil is more favorable for the growth of microörganisms than dry soil. Pasteur, Koch, Tomasi, Crudeli, Tyde and Nicolaier, from 1877 to 1886 were finding different pathogens in the soil, and only Fodor, Dehérain and a few others were studying the microörganisms as to their function in the soil itself.

Koch (20) was the first to point out in 1881 the fact that the numbers of bacteria in the soil are large, and decrease with an increase in depth. At a depth of about one meter the soil is almost free from bacteria. Proskauer (23) took his samples under absolutely sterile conditions and proved that the bacterial numbers decrease with depth. Beumer (1) was the first to dilute the soil before pouring his plate. He also found a great decrease with depth. Fränkel (13) made the first exact study of bacterial numbers in the soil. He was the first investigator to study virgin soils, not contaminated with sewage. He found a gradual decrease of bacterial numbers with depth of soil, from 90,000-300,000 at the surface, to 100-700 at a depth of 2.5 meters. The change in numbers with depth was not gradual, but sudden and irregular. He found larger numbers in summer than in winter, more in cultivated than in uncultivated soils. He concluded that the season of the year and surface covering have no great influence upon bacterial numbers at different depths.

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Maggiora (22) found a decrease from 69,000,000 bacteria per gram of soil at the surface to 17,000,000 at a depth of 4 meters, and from 32,000,000 at the surface to 18,000 at a depth of 3 meters, but his large numbers are probably due to the fact that he allowed the soil to stand in the laboratory for a few days before examining it. Reimers (24) found a decrease from 2,564,000 organisms at the surface to none at a depth of 6 feet in stoney soil, and from 524,500 at the surface to 5,800 at a depth of 1.5 meters in wet meadow land. Houston (16) found a decrease from 1,680,000 bacteria per gram of soil at the surface to 410 at a depth of 6 feet. Caron (6), Stoklassa and Earnest (27), Kebrehl (18), Chester (7), King (19), Waite and Squires (29), and Brown (2) noted a decrease of bacterial numbers with depth. Chester demonstrated that soils rich in humus show larger numbers of bacteria than those low in humus. When, however, the quantity of humus in the soil is too high, deleterious products, especially those of an acid character, are produced which kill many bacteria and inhibit the development of others. Rich woodland is in this condition and always shows low numbers of bacteria. King (19) noted that the bacterial numbers commencing at the surface increase to a depth of 5 or 6 inches, depending upon the depth of plowing, and disappear at a depth of 7 feet below the surface. He concluded that the periods of maximum and minimum activity are, to a certain extent, independent of moisture and temperature and are possibly due to the presence of bacterial by-products. Brown (2) found the greatest number of organisms, in the different soil types, at a depth of 4 inches. The numbers decreased with depth, the greatest fall occurring within the first 12, and sometimes the first 8 inches.

As to the question of cultivation, most of the investigators seem to agree that soils of the same type contain larger numbers of bacteria when cultivated than when left uncultivated. This is shown by Houston (16), Fabricius and Feilitzen (11), Waite and Squires (29), and Burri (5). These investigators concluded that cultivation increases bacterial activities and available plant food. Fischer (12) found smaller bacterial numbers in a cultivated than in a non-cultivated moor soil. Engberding (10) found that the water content of the soil has an important bearing upon bacterial numbers; cultivation increases the numbers by increasing the water content.

Hiltner and Störmer (15) made a thorough study of cultivated and uncultivated soils and came to the conclusion that with similar types of soil and the same treatment, at the same depth, a unique microflora is found at definite periods. Samples taken under the same conditions gave identical results and possessed similar bacteriological characters; while the soils differing in any respect had different bacteriological relations. However, they believed that cultivation does not increase the

bacterial numbers that are able to grow on gelatin, but rather causes a decrease. Temple (28) found that the addition of cow manure to the soil greatly increases the number of bacteria in the soil and that this increase continues over a considerable period. Conn (9), examining different soils for their different contents, found a difference between 78,000,000 and 4,000,000 per gram. He thought that these differences are only small when compared with the variations of the bacterial counts of milk or water; also that the high and low counts are associated with high and low moisture contents, respectively, rather than with differences in soil type.

Very little attention has been paid to the numbers of microörganisms in the soil during the different seasons of the year. Kossowitz (20) found in several soil samples that he took in the winter that there were smaller bacterial numbers than those found in the same place in the summer, but he does not tell whether or not the soil was frozen when the samplas were taken. Remy (25), taking his soil samples all through the growing season, found that the bacterial numbers depend on the moisture content of the soil, but he took no samples while the soil was frozen. Hiltner and Störmer (15) took several soil samples through the winter, but they did not find any great difference in batcerial numbers in the winter and in the summer. Their results seem to indicate that the numbers depend on the moisture content of the soil. Engberding (9) found that the variation of soil temperature had relatively little influence upon the bacterial numbers, which were rising and falling in the warm part of the year with the water content of the soil; long continued frost seemed to depress the numbers.

Conn (8), after a careful comparison of bacterial numbers in frozen and unfrozen soil, came to the conclusion that the number of bacteria in frozen soil is generally larger than in unfrozen soil, which is true not only of cropped soil, but also of sod and fallow land. This increase in bacterial numbers after freezing is not due to an increase in moisture content, even though in an unfrozen condition the bacterial numbers seemed to increase and decrease parallel to the moisture content of the soil. The increase in frozen soil seems to be due to an actual multiplication of the bacteria, rather than to a mere rise of the organisms from lower depths brought about by mechanical forces alone. Finally, there is the work of Brown and Smith (4), who confirm Conn's results of increased bacterial numbers in frozen soil. They advanced the theory that surface tension exerted by the soil particles on the films of water, the presence of salts in the water, and the concentration of the salts which may occur when the main body of water begins to freeze, all cause the hygroscopic water in soils to remain uncongealed, and consequently bacteria may live in it and multiply to a comparatively large extent.

Weber (30) also found that the action of low temperatures greatly increases the numbers of bacteria. Russell and Hutchinson (26) try to explain this phenomenon by the fact that the low temperatures suppress the protozoan activities in the soil, and for that reason allow the bacteria to multiply to large numbers. Given and Willis (14) obtained the lowest bacterial counts in the latter part of September, when the soil was very cold, but not frozen. Fairly high counts were obtained when the soil was frozen, but these were not the largest counts obtained through the year.

EXPERIMENTAL.

Methods employed. An effort was made to use a medium which would permit of the development of the largest possible number of bacteria. Several media commonly used for quantitative bacteriological work were compared. Brown's "egg-albumen" agar (3) was found to be the best medium for the development of the largest numbers of bacteria. This medium is composed as follows:

1000 c.c. water,
10 gm. dextrose,
.5 gm. K_2HPO_4,
.2 gm. $MgSO_4$,
.1 gm. egg-albumen,
Trace of $Fe_2(SO_4)_3$,
15 gm. agar.

It was also found that .15 gm. of egg-albumen gave better results than .10 gm.; consequently, this medium was adopted in the following work. The egg-albumen was dissolved in a little cold water to which a few drops of NaOH were added, and this was added to the hot medium that was already prepared. If the albumen was dissolved first in a little NaOH it was found that no coagulation of the albumen took place, even upon mixing it with the hot medium. The latter was then tubed and sterilized at 10 pounds pressure for 30 minutes.

The plates were prepared by the usual dilution method. In order to avoid contamination, this procedure was followed: 200 c.c. Erlenmeyer flasks with cotton plugs were sterilized in a drying oven, cooled, and weighed. The soil in the sampling bottle was well mixed, and a portion of it was transferred with a sterilized spatula to the flask. The flask was weighed again and the weight of the transferred soil portion was determined by finding the difference. Sterile water was added to the flask in order to have a volume just ten times the weight of the sample. This gave a dilution of 1-10. The mixture was shaken for 5 minutes. Then the following dilutions were made, using 1 c.c. pipettes for the transfers: 1 c.c. of the infusion added to 199 c.c. of sterile water, gave a dilu-

tion 1-2,000; after shaking it well, 1 c.c. of this dilution was transferred into 99 c.c. of sterile water in the case of surface soil samples, where the bacterial numbers were large, so as to have a dilution 1-200,000. In the case of the subsoil samples 1 c.c. of the 2,000 dilution was transferred into 9 c.c. of sterile water, so as to have the dilution of 1-20,000. Plates were poured in triplicate from the highest dilutions and one plate from the lower dilution to serve as a check. In all cases the results of the highest dilutions made are given. The plates were incubated for six days at 20-22° C., at the end of which time the counts were made. The six-day period of incubation was compared with the three-day period, and since the longer period allowed a greater development of colonies, it was adopted.

Methods of taking soil samples. The instruments used were 24 cylindrical glass flasks, 10 inches high and 3 inches in diameter, plugged with cotton and sterilized; a small alcohol lamp, a knife, a ruler, a thermometer, a rag soaked in alcohol for sterilization of tools, a shovel, and a pickaxe. All precautions were taken to avoid any possible contamination. A pit approximately 12 inches wide, 30 inches long, and 30 to 32 inches deep was dug. With a sterilized knife the soil was removed at each depth prior to sampling. After removing the outside soil, the knife was again sterilized over the alcohol lamp, and the sample transferred to the sterilized bottle, after removal of the cotton plug. The plug was replaced at once, after the soil had been introduced into the bottle. When the soil was frozen, the soil had to be cut out with the pickaxe, which was used for making the pit; in that case the axe was carefully cleaned before sampling, with the rag soaked in alcohol.

The samples were taken to the laboratory, where the inoculations were made at once, uniformity being carefully obtained in making the infusion. In the case of frozen soil, a piece of it was introduced into a sterile flask, and regular treatment followed, the soil thawing out in the process of shaking. When the samples were brought into the laboratory, the bacterial inoculations were made first, and 100 gm. of soil from each sample were transferred into porcelain dishes. These were exposed to the air for a few days, and then weighed again, difference in weight being taken as the percentage of moisture in the original sample.

Soils used. Four soils were used, designated as A, B, C, and D. Soils A, B, and D are classed by the Bureau of Soils as Sassafras loam, and C as Alloway clay loam. The mechanical composition of these soils is shown in the table on the following page.

Plot A is one of the plots of the Botanical Department of the New Jersey Agricultural Experiment Station. This plot has been manured for the last twenty years with 15 to 20 tons of stable manure per acre annually and has received an application of lime every five years. Gar-

den crops, such as peppers, tomatoes, beans, etc., have been grown on this plot every year without any regular rotation.

Plot B is the unfertilized plot of the apple orchard on the College Farm, at the New Jersey Station, near Plot A. The orchard was planted in 1896 for fertilizer experiments. Plot B is the check plot, which did not receive any fertilizer at all, but for the last three years oats have been grown as a cover crop. The orchard is cultivated during the summer from eight to ten times. The growth of the trees in the unfertilized plots is not any different from that of the plots receiving fertilizer every year. This shows that the soil is not poor in plant food, since it can compete for twenty years with fertilized plots in growing apple trees.

SASSAFRAS LOAM.[1]

	Organic Matter	Gravel 2-1 mm.	Coarse Sand 1-.5 mm.	Medium Sand .5-.25 mm.	Fine Sand .25-.1 mm.	Very Fine Sand .1-.05 mm.	Silt .05-.005 mm.	Clay .005-.0001 mm.
Surface	1.45	2.26	8.28	6.30	9.94	10.08	53.38	8.80
Subsoil	.98	1.48	5.80	5.64	10.56	11.34	49.16	15.70

ALLOWAY CLAY LOAM.

Surface	3.46	1.18	3.52	3.80	5.42	5.84	53.80	25.30
Subsoil	1.03	.80	3.96	4.98	6.88	7.10	46.82	28.76

Plot C is a timothy meadow which has been under grass for the last six years, and before that it was under oats and peas, and other forage crops.

Plot D is the wood-lot of the College Farm, which has not been plowed for at least 50 years, if at all.

In soils A, B, and C, in all instances, six samples were taken at depths of 1, 4, 8, 12, 20, and 30 inches, respectively. The first eight samples of Soil D were taken at all six depths, except in three cases, when, because of free water at the lowest depth, the sample was not taken at that point. The last five samples from Soil D were taken at depths of only 1 and 4 inches. A total of thirteen samplings were made, from January 30, 1915, to January 4, 1916.

Table I gives the climatic conditions through the year. The nitrogen content of the soil is found in Table II. This was determined by the Kjeldahl method. The total carbon determinations made by the official method are given in Table III. Table IV shows the lime-requirements of the soils determined by the Veitch method.

[1] This is taken from the "Soil Survey of the Trenton Area, in New Jersey," Bureau of Soils, 1902.

TABLE I.

CLIMATIC CONDITIONS FOR THE YEAR 1915-1916.[1]

Date of Sampling	Air temperature at day of Sampling		Rainfall for the month	Soil temperature			
	Maximum	Minimum	Inches	A	B	C	D
1915	° C.	° C.	° C.	° C.	° C.	° C.	° C.
Jan. 30.....	—2	—12	6.03	[2]—2	[2]—1	1.5	0.5
Feb. 12.....	9	2	5.81	[2]—1.5	[2]0	2	1
Mar. 1.....	3	—6	.75	1	1.5	3	2.5
Mar. 23.....	18	1	.75	6	8	9	8
Apr. 16.....	20	0.5	2.75	10	10	12	11
May 8.....	25	18	3.87	12	11	14	13
June 3.....	16	8	2.66	16	14	20	19
July 7.....	27	12	5.96	22	20	21	23
Aug. 8.....	29.5	18	9.97	20	19	22	23
Sept. 10.....	30.5	21	2.06	24	23	24	21
Oct. 21.....	25	15	2.13	21	20	22	22
Nov. 30.....	6	—2	1.61	[2]0	1	1	2
1916			December				
Jan. 4.....	10	—2	3.90	[2]—0.5	[2]0	1	1

[1] The air temperature and rainfall statistics were taken from the records of the weather bureau stationed at the College Farm.

[2] At the date of sampling, the soil was frozen.

TABLE II.

NITROGEN CONTENT, IN PER CENT, OF THE SOILS AT DIFFERENT DEPTHS.

Soil	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches
A	0.1127	0.1176	0.0973	0.0581	0.0420	0.0581
B	0.1158	0.1071	0.1036	0.0819	0.0413	0.0287
C	0.1918	0.1596	0.1267	0.1050	0.0518	0.0350
D	0.2345	0.1015	0.0469	0.0294	0.0294	0.0307

TABLE III.

CARBON CONTENT, IN PER CENT, OF THE SOILS AT DIFFERENT DEPTHS.

Soil	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches
A	1.56	1.41	1.76	0.57	0.63	0.67
B	0.90	0.41	0.44	0.41	0.18	0.17
C	1.70	1.12	1.05	0.53	0.20	0.20
D	3.38	1.58	0.56	0.23	0.09	0.12

TABLE IV.

LIME-REQUIREMENT, IN POUNDS, OF THE SOILS AT DIFFERENT DEPTHS.

Soil	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches
A	200	100	0	0	0	0
B	2500	2000	1800	1600	800	1000
C	500	500	600	500	600	400
D	4600	3400	2400	2100	1800	1600

When the data presented in Table V are examined, one sees that in all cases the bacteria decreased with depth, except for the first four inches. In some cases the highest numbers were found at a depth of 1 inch from the surface, while in other cases the numbers increased from depths of 1 to 4 inches, then decreased regularly. On the average, the

TABLE V.

NUMBER OF BACTERIA PER GRAM OF AIR-DRIED GARDEN SOIL—A.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches	Average
1915							
Jan. 30.....	8,700,000	12,420,000	3,440,000	1,986,000	301,000	381,000	4,538,000
Feb. 12.....	5,965,000	6,161,000	5,551,000	1,075,000	759,000	503,000	3,336,000
Mar. 1.....	8,812,000	5,531,000	3,370,000	1,591,000	1,183,000	849,000	3,556,000
Mar. 23.....	4,272,000	5,033,000	3,154,000	1,787,000	400,000	310,000	2,493,000
Apr. 16.....	10,700,000	21,400,000	3,300,000	1,690,000	690,000	606,000	6,398,000
May 8.....	4,760,000	8,410,000	4,150,000	1,050,000	554,000	200,000	3,187,000
June 3.....	6,890,000	5,090,000	4,445,000	2,420,000	2,100,000	410,000	3,559,000
July 7.....	7,760,000	6,220,000	2,810,000	800,000	310,000	300,000	3,033,000
Aug. 8.....	5,000,000	6,670,000	4,000,000	817,000	533,000	420,000	2,907,000
Sept. 10.....	8,767,000	7,100,000	4,150,000	1,217,000	353,000	127,000	3,619,000
Oct. 21.....	5,900,000	6,000,000	5,200,000	1,300,000	550,000	500,000	3,242,000
Nov. 30.....	6,900,000	6,000,000	5,400,000	985,000	456,000	290,000	3,338,000
1916							
Jan. 4.....	9,200,000	4,540,000	3,000,000	340,000	220,000	56,000	2,893,000
Average	7,202,000	7,737,000	3,998,000	1,312,000	624,000	381,000	

highest bacterial numbers were found in the garden soil at a depth of 4 inches; the 1-inch depth gave a slightly smaller average. Below 4 inches the numbers decreased rapidly, the greatest fall occurring between depths of 4 and 8 inches. When the moisture content of the garden soil given in Table VI is compared with the bacterial numbers, one sees that

TABLE VI.

MOISTURE CONTENT, IN PER CENT, OF GARDEN SOIL AT DIFFERENT DEPTHS.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches	Average
1915							
Jan. 30.....	15	12	9	6	7	9	9.67
Feb. 12.....	14	21	22	7	7	10	13.50
Mar. 1.....	13	11.8	8.7	7	7	12	9.93
Mar. 23.....	8	10	9	6	10	12	9.17
Apr. 16.....	9	10	11	8	7	12	9.50
May 8.....	9	12	10	11	11	10	10.50
June 3.....	9	11	10	9	8	11	9.67
July 7.....	9	10	10	7	10	9	9.17
Aug. 8.....	12	12	10	9	12	13	11.33
Sept. 10.....	10	11	9	9	10	11	10.00
Oct. 21.....	9	8	9	7	7	8	8.00
Nov. 30.....	9	9	9	8	10	11	9.33
1916							
Jan. 4.....	19	14	12	8	8	9	11.67
Average	11.15	11.68	10.67	7.85	8.77	10.54	

also in the case of moisture, the content rose from the 1-inch depth to the 4-inch depth, then decreased below the depth of 4 inches. But at depths lower than 12 inches, where the moisture content began to increase, the bacterial numbers decreased regularly. This is easily understood, since the lower depths, probably because of the exclusion of air and plant food, do not favor bacterial development.

The highest bacterial numbers throughout the year were found in Soil A on April 16, when 21,700,000 bacteria were found at a depth of 4 inches, and 10,700,000 one inch from the surface. On January 30, February 12, November 30, and January 4, the soil was frozen to a depth of 6 or 8 inches. The bacterial numbers from the samples taken on those dates are fairly high, but not the highest. In regard to the relation between the bacterial numbers and moisture content through the different seasons of the year, there does not seem to be any close association in the data presented in Tables V and VI.

The bacterial numbers and moisture content of Soil B are given in Table VII and VIII. This soil, which contains a much smaller amount of organic matter than Soil A, as seen from Table III, is as rich in nitrogen as the other soil, giving a narrower carbon-nitrogen ratio than Soil A.

TABLE VII.

NUMBER OF BACTERIA PER GRAM OF AIR-DRIED ORCHARD SOIL—B.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches	Average
1915							
Jan. 30.....	15,430,000	3,590,000	3,000,000	2,202,000	844,000	277,000	4,224,000
Feb. 12.....	6,824,000	4,852,000	3,370,000	1,123,000	1,036,000	468,000	2,945,000
Mar. 1.....	10,422,000	6,897,000	6,519,000	4,556,000	1,068,000	525,000	4,998,000
Mar. 23.....	4,538,000	5,622,000	2,101,000	1,615,000	463,000	753,000	2,515,000
Apr. 16.....	8,250,000	6,170,000	2,030,000	1,650,000	386,000	347,000	3,139,000
May 8.....	6,810,000	3,790,000	2,550,000	1,020,000	990,000	322,000	2,580,000
June 3.....	17,700,000	9,480,000	5,556,000	1,660,000	281,000	210,000	5,814,000
July 7.....	6,230,000	3,700,000	1,010,000	815,000	75,000	52,000	1,960,000
Aug. 8.....	4,833,000	5,000,000	1,867,000	810,000	367,000	340,000	2,203,000
Sept. 10.....	7,600,000	6,900,000	3,250,000	1,500,000	156,000	60,000	3,244,000
Oct. 21.....	5,800,000	6,500,000	2,270,000	1,270,000	720,000	560,000	2,853,000
Nov. 30.....	5,800,000	4,350,000	2,500,000	1,010,000	620,000	540,000	2,470,000
1916							
Jan. 4.....	7,100,000	5,650,000	1,200,000	620,000	110,000	60,000	2,457,000
Average	8,257,000	5,577,000	2,863,000	1,527,000	547,000	347,000	

The lime-requirement of Soil B is also higher than that of A. When the bacterial numbers are compared, one finds in Soil B the highest numbers just below the surface. This is probably due to the fact that the land is always shaded and the first inch of soil is not so dry as that of Soil A. The bacterial numbers decrease rapidly and regularly with depth, the greatest fall occurring between the depths of 1 to 4 and 4 to 8 inches. The highest bacterial numbers for this soil were found June 7. Also in

Soil B the frozen samples contained fairly large numbers of bacteria, but not the highest throughout the year.

TABLE VIII.

MOISTURE CONTENT, IN PER CENT, OF ORCHARD SOIL AT DIFFERENT DEPTHS.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches	Average
1915							
Jan. 30.....	14	11	11	10	6	11	10.50
Feb. 12.....	15	19	19	11	8	9	13.50
Mar. 1.....	12.6	13	10	10	7	10	10.20
Mar. 23.....	9	10	11	9	5	8	8.67
Apr. 16.....	9.5	10.5	11.5	11	5	8	8.58
May 8.....	9	12	11	8	8	9	9.50
June 3.....	11	10	11	10	11	12	10.83
July 7.....	9	10	11	10	9	9	9.67
Aug. 8.....	11	11	10	8	9	10	9.83
Sept. 10.....	8	10	10	7	5	8	8.00
Oct. 21.....	9	10	9	7	5	6	7.67
Nov. 30.....	10	11	11	11	8	9	10.00
1916							
Jan. 4.....	22	15	14	8	11	11	13.50
Average	11.47	11.73	11.50	9.23	7.46	9.23	

It is seen from Table I° that the bacterial numbers in Soil C vary with depth in a manner similar to those in Soil B, the largest numbers occurring at a depth of 1 inch, the numbers then decreasing rapidly with depth. This soil gave, on the average, higher bacterial numbers than the other two

TABLE IX.

NUMBER OF BACTERIA PER GRAM OF AIR-DRIED MEADOW SOIL—C.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches	Average
1915							
Jan. 30.....	6,267,000	2,325,000	1,609,000	556,000	206,000	198,000	1,860,000
Feb. 12.....	9,577,000	2,566,000	2,588,000	1,008,000	427,000	531,000	2,783,000
Mar. 1.....	9,478,000	5,926,000	3,176,000	1,132,000	232,000	162,000	3,351,000
Mar. 23.....	8,607,000	6,904,000	5,259,000	1,682,000	172,000	167,000	3,798,000
Apr. 16.....	11,940,000	4,150,000	2,680,000	1,090,000	220,000	172,000	3,375,000
May 8.....	11,640,000	8,190,000	1,220,000	1,110,000	1,100,000	704,000	3,994,000
June 3.....	5,990,000	4,850,000	1,580,000	823,000	300,000	235,000	2,296,000
July 7.....	6,340,000	5,200,000	3,800,000	1,106,000	100,000	70,000	2,770,000
Aug. 8.....	9,417,000	5,200,000	4,367,000	617,000	237,000	89,000	3,321,000
Sept. 10.....	10,120,000	8,500,000	4,400,000	850,000	356,000	170,000	4,066,000
Oct. 21.....	19,250,000	9,420,000	2,500,000	1,520,000	1,250,000	400,000	5,723,000
Nov. 30.....	10,500,000	6,500,000	2,670,000	980,000	100,000	110,000	3,477,000
1916							
Jan. 4.....	12,600,000	5,120,000	1,200,000	620,000	110,000	60,000	3,285,000
Average	10,133,000	5,758,000	2,850,000	1,007,000	370,000	236,000	

soils, probably because of the higher nitrogen and organic matter content, and high moisture content, connected with moderate acidity. The largest bacterial numbers were found in this soil October 21. At no

period of sampling has this soil been frozen more than 1 inch deep, because of the heavy sod covering the ground, so that the conditions of soil freezing cannot be taken into account.

TABLE X.

MOISTURE CONTENT, IN PER CENT, OF MEADOW SOIL AT DIFFERENT DEPTHS.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches	Average
1915							
Jan. 30.....	25	17	13	16	16	9	16.00
Feb. 12.....	29	17	15	12	11	12	16.00
Mar. 1.....	17	19	19	16.6	17	18	17.77
Mar. 23.....	21	17	15	15	11	12	15.17
Apr. 16.....	19	15	13	14	17	15	15.50
May 8.....	13	13	13	13	16	13	13.50
June 3.....	11	12	12	11	14	15	12.50
July 7.....	17	14	12	12	13	14	13.67
Aug. 8.....	20	14	14	14	12	17	15.17
Sept. 10.....	15	14	13	11	11	12	12.67
Oct. 21.....	19	14	13	12	10	11	13.17
Nov. 30.....	18	19	11	11	16	17	15.33
1916							
Jan. 4.....	29	20	16	13	16	17	18.50
Average	19.46	15.77	13.77	13.12	13.85	14.00	

Soil D, though high in organic matter and nitrogen, especially in the upper four inches of soil, contains small numbers of bacteria. This is probably to be looked for in the high acid content of the soil and the undecomposed condition of its organic matter. As is shown elsewhere, this soil contains large numbers of fungi, and the fungus flora is more extensive than the bacterial flora. This is in accord with other investiga-

TABLE XI.

BACTERIA PER GRAM OF AIR-DRIED FOREST SOIL.—D.

Date of Sampling	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches
1915						
Jan. 30.....	2,778,000	1,058,000	609,000	429,000	156,000	.....
Feb. 12.....	1,847,000	1,658,000	542,000	486,000	230,000	150,000
Mar. 1.....	3,219,000	701,000	338,000	361,000	139,000	.....
Mar. 23.....	2,155,000	805,000	468,000	144,000	232,000	.....
Apr. 16.....	550,000	470,000	500,000	330,000	133,000	116,000
May 8.....	2,120,000	1,940,000	667,000	445,000	345,000	156,000
June 3.....	734,000	785,000	459,000	237,000	80,000	74,000
July 7.....	1,004,000	340,000	270,000	60,000	40,000	23,000
Aug. 8.....	4,233,000	1,820,000	.....	.....	.....	.....
Sept. 10.....	1,800,000	1,120,000	.....	.....	.....	.....
Oct. 21.....	900,000	890,000	.....	.....	.....	.....
Nov. 30.....	2,640,000	1,780,000	.....	.....	.....	.....
1916						
Jan. 4.....	3,170,000	1,870,000	.....	.....	.....	.....
Average	2,088,000	1,172,000	482,000	311,000	169,000	104,000

tions, that moor, forest, and other acid soils, with a high amount of undecomposed organic matter, are poor in bacterial numbers, but contain a rich fungus flora.

TABLE XII.

MOISTURE CONTENT, IN PER CENT, OF FOREST SOIL AT DIFFERENT DEPTHS.

Date of Sampling	1 in.	4 in.	Avg.	8 in.	12 in.	20 in.	30 in.	Avg.
1915								
Jan. 30.....	28	18	23.0	14	15	14	15	17.33
Feb. 12.....	28	24	26.0	17	15	16	20	20.00
Mar. 1.....	42	23	32.5	15	15	23	..	23.60
Mar. 23.....	29	23	26.0	13	12	11	16	17.33
Apr. 16.....	27	15	21.0	13	12	15	14	16.00
May 8.....	25	16	20.5	12	11	15	15	15.67
June 3.....	22	15	18.5	11	11	13	14	14.33
July 7.....	25	20	22.5	11	13	11	12	15.33
Aug. 8.....	24	19	21.5	..	..	..	..	.....
Sept. 10.....	23	11	17.0	..	..	..	..	.....
Oct. 21.....	18	13	15.5	..	..	..	..	.....
Nov. 30.....	30	22	26.0	..	..	..	..	.....
1916								
Jan. 4.....	37	26	31.5	..	..	..	..	.....
Average	27.54	18.85		13.25	13.00	14.75	15.14	

Considering now the results obtained from all the four soils, one finds that the conditions are quite uniform in relation to depth. Soil A, receiving large applications of stable manure and lime, has almost a neutral reaction, has its highest nitrogen and organic matter content at depths of

TABLE XIII.

GENERAL AVERAGES FOR BACTERIAL NUMBERS IN RELATION TO DEPTH.

Soil	1 inch	4 inches	8 inches	12 inches	20 inches	30 inches
A	7,202,000	7,737,000	3,998,000	1,312,000	624,000	381,000
B	8,257,000	5,577,000	2,863,000	1,527,000	547,000	347,000
C	10,133,000	5,758,000	2,850,000	1,007,000	370,000	236,000
D	2,088,000	1,172,000	482,000	311,000	169,000	104,000

4 and 8 inches respectively (the fact that the highest organic content is at a depth of 8 inches is probably due to the fact that decomposition does not go on so rapidly at that depth as in the upper layer), and has the highest bacterial counts 4 inches from the surface. The other three soils, which received no application of manure, and were shaded most of the time, have the highest nitrogen, organic matter, and bacterial contents at a depth of 1 inch, or just below the surface, the numbers regularly decreasing with depth. This shows that not only the soil type and fertilization, but also the crops used have an important bearing upon the bacterial numbers. As to the lime requirements at the different soil depths, the highest lime requirement was found in all soils to be just below the sur-

face, the acidity decreasing regularly with the depth, the meadow soil having an almost uniform lime-requirement from the surface down to a depth of 30 inches.

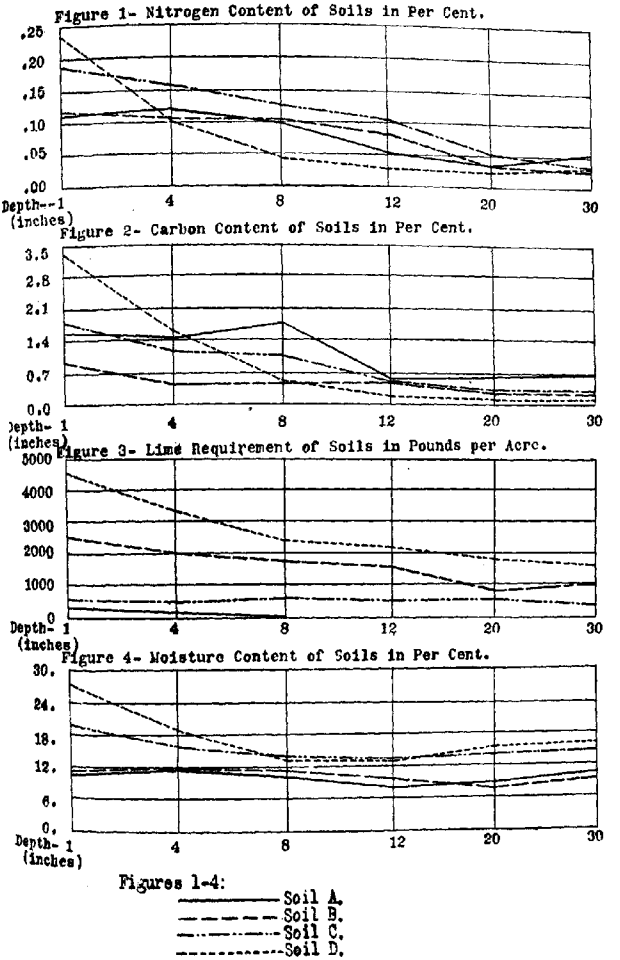

Fig. 1-4. Content of nitrogen, carbon, lime and moisture in the four types of soil used.

The moisture content of the soils is highest 4 inches from the surface in the garden and orchard soils, and 1 inch from the surface in the meadow and forest soils. The moisture content decreases with depth to 12 or 20 inches below the surface; then it begins to increase.

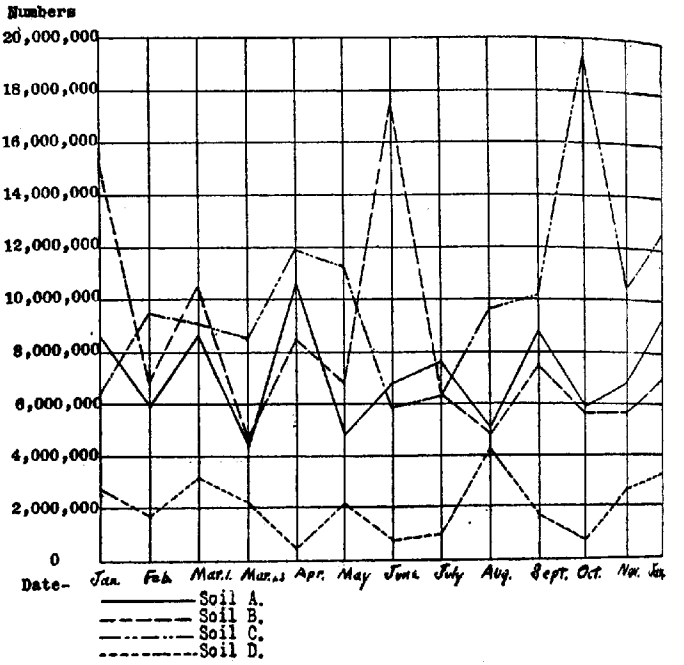

Fig. 5. Numbers of bacteria at the depth of 1 inch throughout the year.

The results, under the conditions at hand, with the culture media used, do not seem to confirm the conclusions of some investigators that the bacterial numbers are in direct relationship with the moisture content of the soil. The moisture, as well as the temperature, seem to have a bearing upon the numbers, but the changes in bacterial numbers cannot be explained by any of these conditions. For, in addition to the temperature and the moisture, there are so many influencing factors, such as soil type, soil treatment, crops used, condition of the organic matter of the soil, and soil reaction, that all of them have to be taken into consideration for the explanation of changes in the microörganic activities in the soil.

When one compares the changes in bacterial numbers in the different times of the year, he finds different results with the various soils used in this investigation. Soil A contained the highest numbers April 16, B—June 3, C—October 21, D—August 8. The frozen soil, though containing high bacterial counts, did not give the largest numbers found through the year. The variation of these results from those of Conn (7) and Brown (4) are to be looked for in the character of the soil, and the length of time during which the soils were frozen. As seen from the results of Brown's (4) investigation, the soil that he used was already

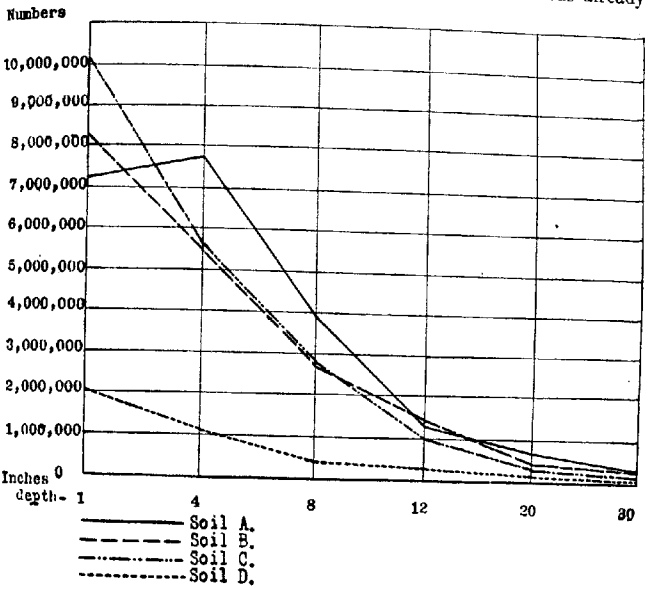

Fig. 6. Numbers of bacteria in different depths of soil: average for whole year.

frozen January 11, but he still found low bacterial numbers on that date, as well as on January 26 and February 11; and only on March 1, after the soil had been frozen almost two months, did he find an increase in bacterial numbers. The soils used in this experiment were at no time frozen continually for such a long period, and two of them (C and D) were never found to be frozen at all, when the samples were taken. The differences in the chemical and mechanical composition, soil type and treatment, and climatic conditions will probably account for the difference in the results.

SUMMARY.

1. The greatest number of bacteria were found at a depth of 1 inch in the soils that are under shade all the year round. The garden (A) soil gave on the average the largest numbers 4 inches from the surface.

2. There was a regular decrease in numbers of organisms from a depth of 1 inch (or 4 inches in the case of Soil A) down to a depth of 30 inches.

3. The greatest decrease in numbers between any two consecutive depths of sampling occurred between the 1st and the 4th, or the 4th and the 8th inches.

4. The meadow soil (C) gave the largest bacterial counts at a depth of 1 inch of all the soils, the 1-inch layer of this soil being richer also in organic matter and nitrogen content than that of soils A and B.

5. The forest soil (D), though showing a high carbon and nitrogen content, gave the lowest bacterial counts probably because of the high acidity and large amount of undecomposed organic matter.

6. The numbers of bacteria in the soils studied were not governed either by the moisture content of the different soils, or the nitrogen and carbon contents.

7. There was a gradual decrease in the lime-requirement of the soils from the surface down to a depth of 30 inches, except in the meadow soil.

8. There was also a more or less gradual decrease in the nitrogen and carbon content of the different soils from the surface down to a depth of 30 inches. As an exception, one finds Soil A, where the nitrogen content 4 inches below the surface was higher than at a depth of 1 inch. This is in accord with the increase in bacterial numbers and moisture content of that soil. Perhaps the moisture content, together with the humus and carbon content of the soil combined with its acidity, might account for the variations in bacterial numbers.

9. Frozen soil, though showing a high bacterial content, did not give the largest bacterial numbers through the year. This may be due to the fact that the soils under study have never been frozen for a longer period than 8 or 10 days.

10. The time of maximum bacterial numbers during the year varied with the different soils throughout the year; no two soils showed their maximum bacterial content at the time of any one sampling.

In conclusion, the author wishes to express his sincere thanks to Dr. J. G. Lipman for the helpful suggestions in outlining this work, and to Mr. R. E. Curtis for the assistance in taking the samples.

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THE INOCULATION AND INCUBATION OF SOIL FUNGI.

By NICHOLAS KOPELOFF, *Research Fellow, Rutgers College.*

The attention of soil biologists has recently been directed to a group of organisms, namely soil fungi, the activities of which may prove of no inconsiderable importance in the problems of soil fertility. It has been assumed from time to time, as for example in the protozoan theory advanced by Russell and Hutchinson (14) that ammonia production may be regarded as serving in some degree as an index to soil fertility. Undoubtedly this has likewise been the basis for what little experimentation has heretofore been recorded on the production of ammonia by soil fungi. Marchal (12), Müntz and Coudon (13), McLean and Wilson (11), Waksman and Cook (15) and Coleman (2), have presented data which point definitely to the fact that many soil fungi are capable of producing more ammonia from organic nitrogenous compounds than even the most efficient bacterial ammonifiers, such as those belonging to the *Mycoides* group. Presumably, the methods employed in soil bacteriology will be adapted, with the necessary modifications, to the study of the activities of soil fungi. However, a certain amount of preliminary data concerning fundamentals in methodology is prerequisite to further investigation in this branch of soil biology and it is in this spirit that the following experiments were planned.

In general, the procedure in ammonification studies with pure cultures of bacteria and fungi have been identical. It is unnecessary at this time to advance the evidence justifying the adoption of soil as a medium in the study of soil biology problems; suffice it to say that it has been so widely accepted as to warrant its use in this and similar experimentation. The organic nitrogenous materials most commonly employed are of animal and of vegetable origin, dried blood and cottonseed meal, respectively. The method followed by the most recent investigators regarding the inoculating materials, is to use a measured quantity of spores of the organism concerned.

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[1] Part I of thesis submitted in partial fulfilment for the degree of M.Sc.

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I. INOCULATION STUDIES WITH CERTAIN SOIL FUNGI.

METHODS.

In the experiments to be considered presently, the inoculation material was prepared as follows: One-hundred cubic centimeter portions of Cook's No. II fungi medium which contains

Water	1000 c.c.
Glucose	20 gm.
Peptone	10 gm.
K_2HPO_4	0.25 gm.
$MgSO_4$	0.25 gm.

were sterilized in 250-c.c. Erlenmeyer flasks and a few spores, from a pedigree culture of the fungus to be studied, introduced into the medium. It was then incubated at room temperature long enough to allow an abundance of spores to appear on the growth of mycelia which covered the surface of the liquid. The period of time necessary for such spore formation varies from 7 to 14 days, depending upon the individual organism. In order to get the spores distributed throughout the liquid, the flask is whirled for about ten minutes. In the case of *Penicillium* and *Zygorrhyncus* it was advisable to scrape the surface growth with a sterile platinum needle, in order to increase the number of spores which would otherwise be quite scanty. The liquid containing the spores in suspension was then transferred to a sterile flask for the purpose of leaving behind as much of the mycelia as possible, because of the fact that the latter clogs the pipette which is used subsequently to deliver the inoculum.

The soil used was Norfolk sandy loam having a lime requirement of 2,300 pounds CaO per acre, which is a favorable reaction for the activities of soil fungi, as reported in Part II of this thesis (8). One hundred fifty-five mg. N. in the form of dried blood and cottonseed meal, respectively, were added to 100-gm. portions of the soil, which were then made up to optimum moisture content by the addition of 13 c.c. of water and 3 c.c. for each gram of organic matter used. Proper deduction was made for the different amounts of inoculum added. The 200-c.c. cotton-plugged Erlenmeyer flasks containing soil were sterilized in the autoclave at 15 pounds pressure for 15 minutes. This process is, of course, responsible for the release of some additional available nutrients (1). After allowing the soil to become cool it was inoculated with the desired amount of spore-suspension by means of a sterile pipette. The necessary precautions against contamination must be strictly observed in such manipulation. The flask containing the inoculum was whirled thoroughly prior to each pipetting to ensure an even distribution of spores. Qualitative tests were carried out on Lipman and Brown's modified synthetic agar (10) for

bacterial contamination. The flasks containing soil were incubated for 7 days at 20° to 22° C., at the end of which time the soil was transferred to copper flasks, and ammonia distilled according to the magnesium oxide method, titrating with tenth normal acid and alkali.

The amounts of inoculum added in both series of organic matter were 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 3.0, 4.0 and 5.0 c.c. respectively. In order to ascertain precisely the number of spores which were present in each of the units mentioned above, a count was made with each fungus for the number of spores per 1 c.c. of spore-suspension. The apparatus used for counting blood corpuscles (Blutkörperzählapparat) employed by Kopeloff, Lint and Coleman (9) in the counting of soil protozoa was adapted to this purpose. Since the fungus spores have, comparatively speaking, no motility, the experimental error of 5 per cent found previously would be somewhat reduced in the present determinations.

EXPERIMENTAL.

The fungi employed were isolated in pure pedigree culture from soil on the College Farm. Goddard (6), Jensen (7), and Dale (4, 5) make mention of some of these organisms, which with the assistance of Mr.

TABLE I.

INOCULATION—PENICILLIUM SP. 10.

Lab No.	Spores c.c.	Organic Matter	H_2O c.c.	Mg.N.	Mg. N.	Av.Mg.N.	Increase over ch'k Mg. N.	No. of Spores
		155 Mg.N.						
131-132	0.2	Dried Bld.	16.5	6.70	6.80	6.75	3.45	19,200
133-134	0.4	"	16.3	8.83	9.31	9.07	5.77	38,400
135-136	0.6	"	16.1	14.82	16.21	15.52	12.22	57,600
137-138	0.8	"	15.9	15.30	12.90	14.10	10.80	76,800
139-140	1.0	"	15.7	15.92	15.92	15.92	12.62	96,000
141-142	2.0	"	14.7	21.51	21.51	21.51	18.21	192,000
143-144	3.0	"	13.7	24.70	22.50	23.60	20.30	288,000
145-146	4.0	"	12.7	26.98	27.42	27.20	23.90	384,000
147-148	5.0	"	11.7	27.84	26.37	27.21	23.91	480,000
		155 Mg.N. Cot'nseed						
149-150	0.2	Meal	20.3	9.78	10.61	10.20	6.40	19,200
151-152	0.4	"	20.1	12.73	12.28	12.51	8.71	38,400
153-154	0.6	"	19.9	12.19	13.60	12.90	9.10	57,600
155-156	0.8	"	19.7	16.86	16.74	16.80	13.00	76,800
157-158	1.0	"	19.5	21.18	21.83	21.51	17.71	96,000
159-160	2.0	"	18.5	24.86	24.03	24.45	20.65	192,000
161-162	3.0	"	17.5	28.91	29.00	28.96	25.16	288,000
163-164	4.0	"	16.5	25.80	26.60	26.20	22.40	384,000
165-166	5.0	"	15.5	33.00	32.01	32.51	28.71	480,000

S. A. Waksman were identified as follows: *Rhizopus Oryzae* (Wendt), *Zygorrhyncus Vuilleminii* (Namyslowski), *Rhizopus nigricans* (Ehrenberg), *Penicillium* sp. 10.[1] It should be noted that these identifications

[1] This organism appears to be identical with a member of Group 10 of soil *Penicillia* studied by S. A. Waksman, the description of which will appear at a later date.

are open to question, since time did not permit of corroboration by mycological specialists.

Considering first the effect of inoculating various quantities of spores of *Penicillium* sp. 10, as recorded in Table I and graphically illlustrated in figure 1, it will be observed that there were present 96,000 spores per 1 c.c. of inoculum. Thus the range from 0.2 to 5.0 c.c. represents the gradation in numbers of spores from 19,200 to 480,000. It will be observed that in the dried blood series there is, with one exception, a gradual increase in ammonia accumulation with increasing quantities of inoculum

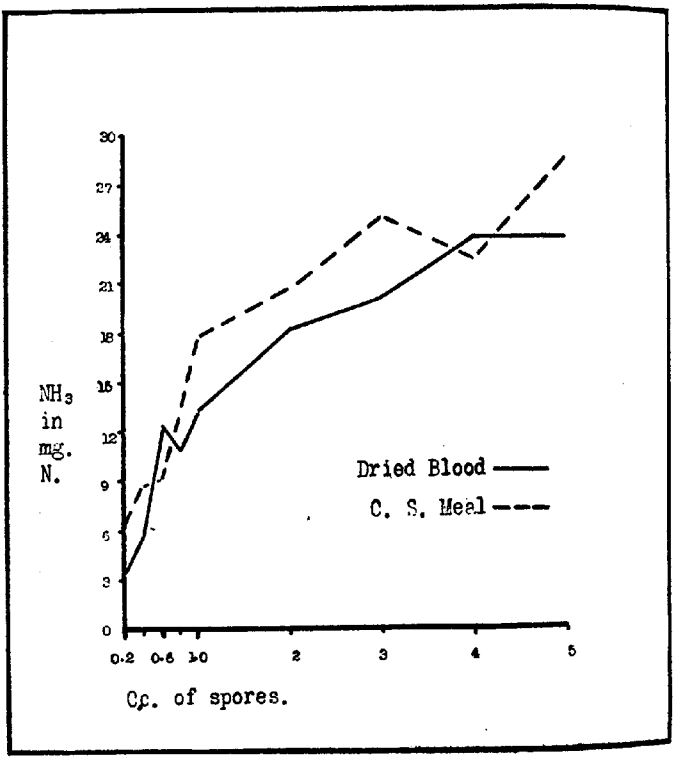

Fig. 1. Inoculation of *Penicillium sp.* 10. Increase over check of ammonia in mg. N.

up to 4 c.c., which represents 384,000 spores. The increase is even more perceptible in the cottonseed meal series (with the exception of 4 c.c., where there is a depression for some unaccountable reason). Thus it

appears that with this organism the number of spores present directly influences the amount of ammonia accumulated. However, the increase in ammonia is not as great as the increase in spores, for where there is a 500 per cent increase of the latter, the corresponding increase in ammonia is in general about 150 per cent, or only one-third as great. There is, then, good reason to believe that only a limited number of spores become effective in influencing ammonia accumulation. That this is largely due

TABLE II.

INOCULATION—RHIZOPUS NIGRICANS.

Lab. No.	Spores c.c.	Organic Matter	H_2O c.c.	Mg. N.	Mg. N.	Av. Mg. N.	Increase over ch'k Mg. N.	No. of Spores
		155 Mg. N.						
70-71	0.2	Dried Bld.	16.5	19.53	17.84	18.69	15.39	79,200
72-73	0.4	"	16.3	26.94	26.94	26.94	23.64	158,400
74-75	0.6	"	16.1	21.38	23.69	22.54	19.24	237,600
76-77	0.8	"	15.9	28.91	28.76	28.84	25.54	316,800
78-79	1.0	"	15.7	29.07	27.39	28.23	24.93	396,000
200-201	1.0	"	15.7	38.14	35.76	36.95	33.65	700,000
202-203	2.0	"	14.7	41.06	41.05	41.06	37.76	1,400,000
204-205	3.0	"	13.7	38.76	39.07	38.92	35.62	2,100,000
206-207	4.0	"	12.7	39.45	42.30	40.88	37.58	2,800,000
208-209	5.0	"	11.7	50.02	51.00	50.51	47.21	3,500,000
		155 Mg. N. Cot'nseed						
80-81	0.2	Meal	20.3	36.07	34.76	35.42	31.62	79,200
82-83	0.4	"	20.1	33.88	36.94	35.41	31.61	158,400
84-85	0.6	"	19.9	39.45	37.57	38.51	34.71	237,600
86-87	0.8	"	19.7	38.79	37.83	38.31	34.51	316,800
88-89	1.0	"	19.5	39.80	39.90	39.85	36.05	396,000
210-211	1.0	"	19.5	41.65	43.74	42.70	38.90	700,000
212-213	2.0	"	18.5	41.97	42.88	42.43	38.63	1,400,000
214-215	3.0	"	17.5	41.60	42.76	42.18	38.38	2,100,000
216-217	4.0	"	16.5	53.41	49.52	51.47	47.67	2,800,000
218-219	5.0	"	15.5	54.95	57.35	56.15	52.35	3,500,000

to a question of food supply is probable, since it is apparent that the conditions are hardly favorable for the complete utilization of the organic nitrogen present. Again, limited germination due to the operation of other factors might be responsible for the above-mentioned phenomenon. The accumulation of ammonia as measured in such experimentation represents the resultant of the two concomitant factors of production and consumption, consequently complexity must be anticipated.

From the data presented in Table II and its graphic representation in figure 2, it will be noted that the inoculation of spores of *Rhizopus nigricans* in increasing amounts effects, in a general way, an increase in the amount of ammonia accumulated. There are several exceptions to be noted in the dried blood series, while in the cottonseed meal series the gradations are not sharply defined. However, the tendency towards parallel increase is evident when one compares the ammonia produced

upon the inoculation of 0.2 c.c., representing 79,200 spores, and of 1.0 c.c., representing 396,000 spores. In the dried blood series, the former is 15.39 mg. of nitrogen compared with 24.93 mg. of nitrogen, or an increase of about 62 per cent; in the cottonseed meal series it is 31.62 as compared to 36.05 mg. of nitrogen, or an increase of about 14 per cent.

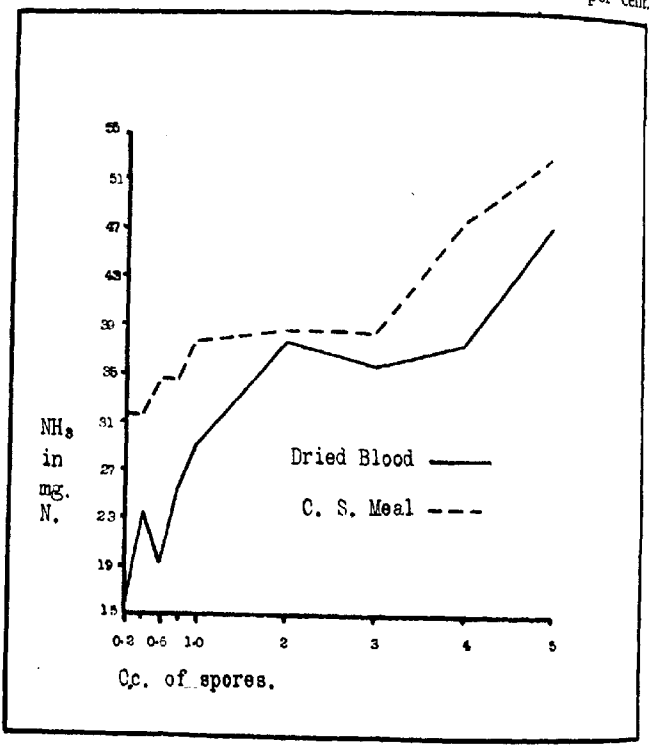

Fig. 2. Inoculation or *Rhizopus nigricans*. Increase in ammonia over check in mg. N.

Comparing the ammonia accumulated when 1 c.c. of inoculum representing 700,000 spores is used as against 5 c.c. or 3,500,000 spores, in the dried blood series there is an increase from 33.65 to 47.21 mg. of nitrogen, or about 40 per cent, whereas in the cottonseed meal series there is an increase from 38.90 to 52.35 mg. of nitrogen, or about 35 per cent.

It will be observed that two quantities of spores are recorded as present in 1 c.c. of inoculum. This is a result of having performed the inoculation of 0.2 to 1.0 c.c. at a different time from the inoculation of 1 to

5 c.c. However, this only seems to indicate more emphatically, perhaps, that the number of spores added has a direct influence on ammonia accumulation. For in the case of dried blood, the difference in ammonia between inoculation with 396,000 as compared with 700,000 spores is 8.8 mg. of nitrogen, or about 33 per cent increase. With cottonseed meal, the difference is comparatively insignificant, being only 3 mg. of nitrogen, or 8 per cent increase. Again, it appears that there is no evidence of a direct quantitative proportion obtaining between the increase in the number of spores added and an increase in ammonia accumulation. However, despite the lack of entire consistency in the results, there is a strong indication that with with *Rhizopus nigricans* an increase in the number of spores added, is accompanied by an increase in ammonia accumulation with both of the organic nitrogenous materials employed.

TABLE III.

INOCULATION—ZYGORRHYNCUS VUILLEMINII.

Lab. No.	Spores c.c.	Organic Matter	H_2O c.c.	Mg. N.	Mg. N.	Av. Mg. N.	Increase over ch'k Mg. N.	No. of Spores
		155 Mg. N.						
90-91	0.2	Dried Bld.	16.5	9.23	8.92	9.08	5.78	15,200
92-93	0.4	"	16.3	9.38	9.38	9.38	6.08	30,400
94-95	0.6	"	16.1	8.92	8.92	8.92	5.62	45,600
96-97	0.8	"	15.9	9.23	9.38	9.31	6.01	60,800
98-99	1.0	"	15.7	11.68	10.30	10.99	7.69	76,000
220-221	1.0	"	15.7	9.23	9.54	9.39	6.09	100,000
222-223	2.0	"	14.7	10.32	10.23	10.28	6.98	200,000
224-225	3.0	"	13.7	10.46	10.61	10.54	7.24	300,000
226-227	4.0	"	12.7	10.77	10.36	10.57	7.27	400,000
228-229	5.0	"	11.7	11.84	11.84	11.84	8.54	500,000
		155 Mg. N. Cot'nseed						
100-101	0.2	Meal	20.3	27.68	26.22	26.95	23.15	15,200
102-103	0.4	"	20.1	28.61	28.15	28.38	24.58	30,400
104-105	0.6	"	19.9	27.84	27.53	27.69	23.89	45,600
106-107	0.8	"	19.7	28.30	28.19	28.25	24.45	60,800
108-109	1.0	"	19.5	28.45	28.15	28.30	24.50	76,000
230-231	1.0	"	19.5	31.99	31.68	31.84	28.04	100,000
232-233	2.0	"	18.5	33.68	32.91	33.30	29.50	200,000
234-235	3.0	"	17.5	35.68	35.53	35.61	31.81	300,000
236-237	4.0	"	16.5	35.99	35.68	35.84	32.04	400,000
238-239	5.0	"	15.5	54.37	53.80	54.09	50.29	500,000

From the results recorded in Table III and figure 3, it will be observed that *Zygorrhyncus Vuilleminii* is ordinarily a poor ammonifier of dried blood. Where 1 to 5 c.c. of inoculum were employed however, there is a distinct increase in ammonia accumulation, the lower amounts giving no such definite indication. The same phenomenon is to be noted in the cottonseed meal series. In the latter series it will be seen that when 1 c.c. of inoculum contained 76,000 spores there were 24.50 mg. of nitrogen, while the inoculation of 100,000 spores accounted for an increase

up to 28.04 mg. of nitrogen. The dried blood series furnishes an exception to this rule. This might be explained, together with the rather uncertain results obtained by inoculating 0.2 to 1.0 c.c., upon the basis that this organism is especially dependent on its food supply, and only a limited number of its spores become effective unless the environmental conditions are favorable.

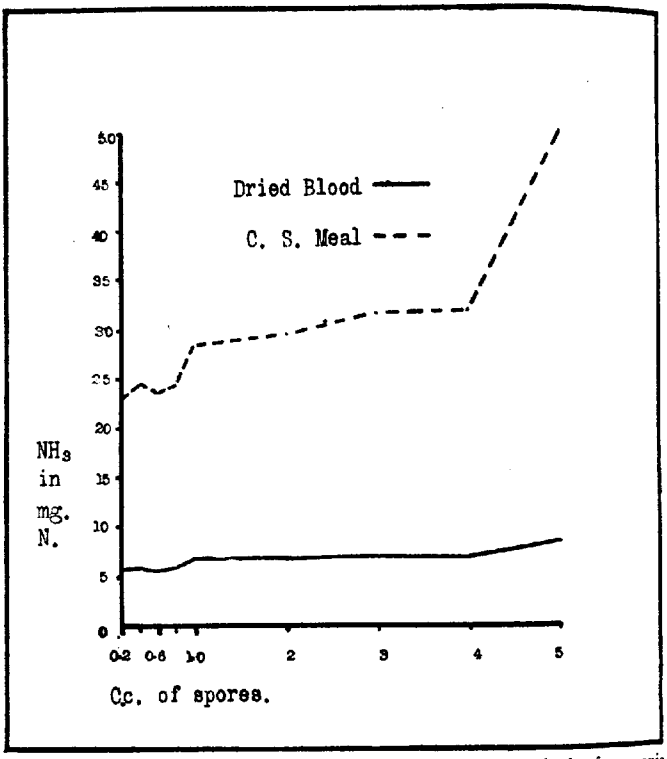

Fig. 3. Inoculation of *Zygorrhyncus Vuilleminii*. Increase over check of ammonia in mg. N.

Comparing the inoculation of 0.2 c.c. representing 15,200 spores with 1.0 c.c. representing 76,000 spores in the dried blood series, there is an increase of ammonia from 5.78 to 7.69 mg. of nitrogen, or about 33 per cent. In the cottonseed meal series there is a negligible increase of 6 per cent. In comparing the inoculation of 1 with 5 c.c. in the dried blood series, there is an increase in ammonia of 40 per cent, and in the cottonseed meal series of 79 per cent. This corroborates the evidence advanced

with *Penicillium* and *Rhizopus nigricans*, that an increase in the number of spores used for inoculation is accompanied by an increase in ammonia accumulation.

TABLE IV.

INOCULATION—RHIZOPUS ORYZAE.

Lab. No.	Spores c.c.	Organic Matter	H_2O c.c.	Mg. N.	Mg. N.	Av. Mg. N.	Increase over ch'k Mg. N.	No. of Spores
		155 Mg. N.						
1-2	0.2	Dried Bld.	16.5	19.81	19.07	19.44	16.14	83,200
3-4	0.4	"	16.3	22.24	23.07	22.66	19.36	166,400
5-6	0.6	"	16.1	24.76	25.07	24.92	21.62	249,600
7-8	0.8	"	15.9	24.15	24.76	24.46	21.16	332,800
9-10	1.0	"	15.7	27.53	25.99	26.71	23.41	416,000
11-12	1.0	"	15.7	17.67	17.87	17.77	14.47	136,000
13-14	2.0	"	14.7	19.79	21.10	20.45	17.15	272,000
15-16	3.0	"	13.7	24.24	23.12	23.68	20.38	408,000
17-18	4.0	"	12.7	27.87	25.65	26.71	23.41	544,000
19-20	5.0	"	11.7	24.74	27.87	26.31	23.01	680,000
		155 Mg. N. Cot'nseed						
21-22	0.2	Meal	20.3	34.61	35.22	34.91	31.11	83,200
23-24	0.4	"	20.1	Lost	Lost	.....	.....	166,400
25-26	0.6	"	19.9	34.14	33.84	33.99	30.19	249,600
27-28	0.8	"	19.7	36.36	35.84	36.10	32.30	332,800
29-30	1.0	"	19.5	29.22	35.22	32.22	28.42	416,000
31-32	1.0	"	19.5	34.74	30.12	32.43	28.63	136,000
33-34	2.0	"	18.5	34.23	33.33	33.78	29.98	272,000
35-36	3.0	"	17.5	33.22	31.20	32.21	28.41	408,000
37-38	4.0	"	16.5	38.88	39.08	38.98	35.18	544,000
39-40	5.0	"	15.5	37.06	36.86	36.96	33.16	680,000

In discussing the results obtained with inoculation of *Rhizopus Oryzae* it is necessary to bear in mind that the inoculation of 0.2 to 1 c.c. was carried on at a different time from that of 1 to 5 c.c. In the latter case the spores per 1 c.c. were only one-third as great in number as in the former; thus the two series must be considered separately. Where dried blood was employed, there is a gradual increase (with one exception) in ammonia with an increase in inoculum from 0.2 to 1 c.c. The same is true in the series of inoculations with 1 to 5 c.c. Comparing the inoculation of 0.2 c.c. representing 83,200 spores with 1 c.c. representing 416,000 spores, there is an increase in ammonia from 16.14 to 23.41 mg. of nitrogen, or 45 per cent. Again, where 1 c.c. representing 136,000 spores is compared with 5 c.c. representing 680,000 spores, there is an increase in ammonia from 14.47 to 23.01 mg. of nitrogen, or 59 per cent. It is especially interesting to note that with 1 c.c. representing 136,000 spores there were 14.47 mg. of nitrogen, while with 416,000 spores in 1 c.c. there were 23.41 mg. of nitrogen, thus emphasizing again

the increase in ammonia accumulation, with an increase in spores inoculated. The results of inoculation where cottonseed meal was used are too variable to allow of any definite conclusions.

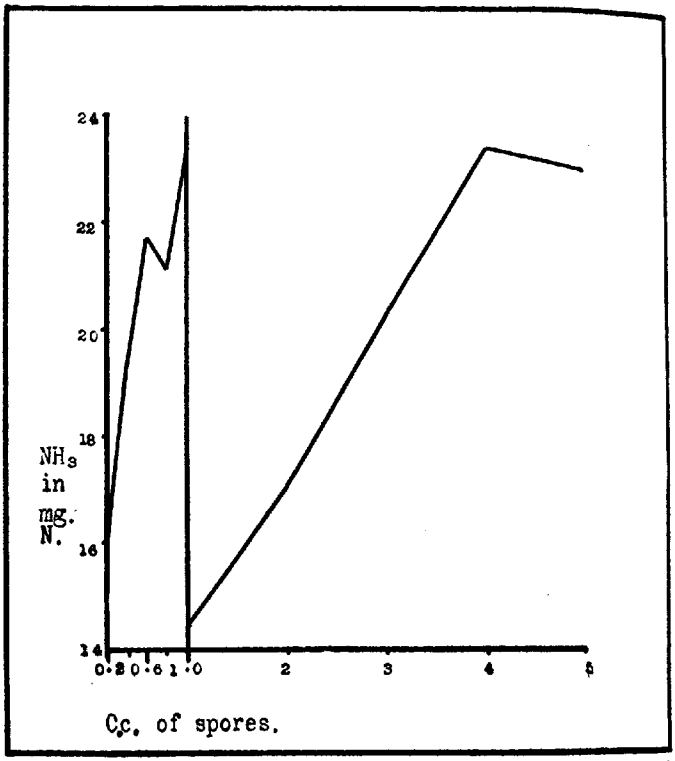

Fig. 4. Inoculation of *Rhizopus Oryzae* in dried blood. Increase over check of ammonia in mg. N.

The data bearing on the point of an increase in ammonia accumulation as a result of an increase in the number of spores inoculated is summarized in Table V. There are presented the ammonification results obtained while using dried blood and cottonseed meal, with 0.2, 1.0 and 5.0 c.c. of spores for inoculation. It may be remarked, parenthetically, that the results for 1 c.c. of inoculation represent an average of the two inoculations performed, whenever these occurred at different times. It will be observed that in all but one instance, an increase in inoculum was responsible for an increase in ammonia.

In order to determine what influence the additions of culture medium *per se* might have, a preliminary test was performed where increasing amounts of Cook's No. II fungi medium were added to 0.2, 1.0 and 5.0 c.c. of inoculum. It was found that 1 c.c. of the medium caused a stimulation in each case, but an increase beyond that amount did not result in increased ammonification. Thus 4 c.c. of medium added to 1 c.c. of spore-suspension gave no increase in ammonia over the addition of only 1 c.c. of medium to 1 c.c. of spore-suspension. Therefore, 5 c.c. of

TABLE V.

COMPARISON OF AMMONIFICATION BY FUNGI, USING 0.2, 1.0 AND 5.0 C.C. OF SPORES FOR INOCULATION.

Fungus	Increase over check Av. Mg. N. Dried Blood Series			Increase over check Av. Mg. N. Cottonseed Meal Series		
	0.2 c.c.	1.0 c.c.	5.0 c.c.	0.2 c.c.	1.0 c.c.	5.0 c.c.
Penicillium sp. 10.....	3.45	12.62	23.91	6.40	17.71	28.71
Rhizopus nigricans.....	15.39	29.24	47.21	31.62	37.48	52.35
Zygorrhyncus Vuilleminii...	5.78	6.89	8.54	23.15	26.27	50.29
Rhyzopus Oryzae.....	16.14	18.94	23.01	31.11	28.53	33.16
Fungus	**Percentage of maximum ammonification with Dried Blood**			**Percentage of maximum ammonification with Cottonseed Meal**		
	0.2 c.c.	1.0 c.c.	5.0 c.c.	0.2 c.c.	1.0 c.c.	5.0 c.c.
Penicillium sp. 10.....	14.4	52.8	100	22.3	61.2	100
Rhizopus nigricans.....	32.6	61.9	100	60.3	71.6	100
Zygorrhyncus Vuilleminii...	68.2	81.2	100	44.4	52.3	100
Rhyzopus Oryzae.....	70.0	82.2	100	88.9	81.4	94.9
Average	46.3	69.5	100	54.0	66.6	98.7

spore-suspension would not carry a sufficient quantity of additional nutrients over that contained in 1 c.c. of spore-suspension, to affect the results seriously. From the lower half of Table V, where are summarized the data on inoculation with 0.2, 1.0 and 5.0 c.c. with regard to the percentage of maximum ammonification, it is evident that 0.2 c.c. caused a production of only 50 per cent of the maximum, while 1 c.c. was responsible for practically 70 per cent. Of course, 5 c.c. yielded the maximum. For all practical purposes, however, and in view of certain other factors to be considered in another connection, it is apparent that 1 c.c. of inoculum gives satisfactory results.

In order to facilitate the discussion of the results in Table V, the same data are presented in a different form in Table VI. The calculations are based upon the percentage increase in ammonia as a result of using 1 c.c. of inoculum compared with 0.2 c.c., and 5 c.c. as compared with 1 c.c. in the dried blood and cottonseed meal series, respectively. In other words, a 500 per cent increase in inoculum has been twice em-

ployed. In the dried blood series (with but one exception) the percentage increase in ammonia, when 1 c.c. is used as against 0.2 c.c., is greater than when 5 c.c. is compared with 1 c.c. The reverse apparently obtains in the cottonseed meal series (with one exception). This might be interpreted as signifying that dried blood is not as acceptable a source of food to these fungi as cottonseed meal, for with an increasing number of spores the competition seems to grow more keen. In point of fact, a casual glance at the relative ammonia accumulation in the tables of results with the different fungi appears to substantiate the observation that with cottonseed meal there is a somewhat greater ammonia accumulation than with dried blood. The last column of Table VI contains a general average of the increase in ammonia due to a 500 per cent increase in inoculum. In general, it might be stated that there was an increase of approximately 45 per cent (except for *Penicillium* which was considerably higher), or, in effect, the increase in inoculum by unit quantities, roughly speaking, increases the ammonia accumulation about one-tenth.

TABLE VI.

PERCENTAGE INCREASE OF AMMONIFICATION BY FUNGI, USING 0.2, 1.0 AND 5 C.C. OF INOCULUM.

Fungus	Dried Blood Series		Cottonseed Meal Series		
	Incr. 1 c.c. over 2.0 c.c. %	Incr. 1 c.c. over 1.0 c.c. %	Incr. 5 c.c. over 0.2 c.c. %	Incr. 5 c.c. over 1.0 c.c. %	Av. In. due to 500% incr. in inoculum
Penicillium sp. 10.....	265	89	177	63	148
Rhizopus nigricans.....	62	40	14	35	38
Zygorrhyncus Vuilleminii	33	[1]6	40	79	51
Rhizopus Oryzae.....	45	59	..	16	40

[1] Not averaged.

The relation of the number of spores used in inoculation to ammonia accumulation has still another important aspect. It does not alone suffice to have determined the amount of inoculum to be used for maximum ammonia accumulation, but it is, moreover, prerequisite to further investigation with soil fungi to establish the optimum amount of inoculum which will bring out the differences which the individual organism manifests in its action upon various materials. Concretely, then, it is desirable to know whether 0.2, 1.0 or 5.0 c.c. for the sake of argument, will produce the greatest difference between the ammonification of dried blood and cottonseed meal respectively.

Some light is thrown upon this point in Table VII. It will be seen that with *Penicillium* 1.0 c.c. of inoculum gives the most noticeable difference between the ammonification of dried blood and cottonseed meal. However, 5 c.c. gives practically the same result. With *Rhizopus nigricans* 0.2 c.c. is superior to 1.0 c.c., which in turn is more striking in its effect than 5 c.c. With *Zygorrhyncus* it will be noted that 1 c.c. is su-

perior to 0.2 c.c., but inferior to 5 c.c. Possibly the poor ammonification of dried blood in this case exaggerates to some extent the large increase of 5 c.c. over 1 c.c. With *Rhizopus Oryzae*, 0.2 c.c. is superior to 1 c.c., which is almost equal to 5 c.c.

Thus considering all four organisms used, it is evident that in a majority of cases 1 c.c. is as efficient as 5 c.c. of inoculum. In effect, this implies that there is no advantage to be derived from increasing the number of spores in the inoculum beyond a certain point. Although the smallest quantity of spores, i. e. 0.2 c.c., was sufficient to bring out the difference between the ammonification of dried blood and cottonseed meal, there are several practical objections to its adoption. 1. Where such a small quantity is used there is an increase in experimental error based upon the technique of manipulation. 2. There is inaccuracy in the use of the pipette. 3. It is more difficult to obtain an equal distribution of spores in such a small sample.

TABLE VII.

DIFFERENCES BETWEEN AMMONIFICATION OF DRIED BLOOD AND COTTONSEED MEAL BY FUNGI, USING 0.2, 1.0, AND 5.0 C.C. OF SPORES FOR INOCULATION.

Fungus	Difference between ammonification of Dried Blood and Cottonseed Meal Av. Mg. N.			No. of Spores per 1 c.c.
	0.2 c.c.	1.0 c.c.	5.0 c.c.	
Penicillium sp. 10	2.95	5.09	4.80	96,000
Rhizopus nigricans	16.23	8.24	5.14	248,000
Zygorrhynchus Vuilleminii	17.37	19.38	41.75	276,000
Rhizopus Oryzae	14.97	9.59	10.15	88,000

In general, 1 c.c is recommended as the most desirable quantity for inoculation for the following reasons. 1. One c.c. gave as striking differences as 5 c.c. 2. It is to be preferred to the latter because of economy of time. 3. Likewise it is preferable because economy of inoculating material. 4. Since less culture medium is added, there is consequently less possibility for the introduction of a disturbing factor.

Thus, in concluding this part of the investigation the salient points which have been established under the conditions of the experiment are as follows:

1. An increase in the number of spores inoculated into the soil is responsible for a proportional increase in ammonia accumulation.

2. Increasing the number of spores used in inoculation beyond a certain point does not further accentuate the difference between the ammonification of dried blood and cottonseed meal by these fungi.

3. One c.c. of spore suspension is the most desirable quantity to employ in soil fungi work.

4. Under the conditions of moisture and temperature employed, cottonseed meal is a more acceptable source of food for these fungi than dried blood.

II.—STUDIES ON THE INCUBATION PERIOD OF ZYGORRHYNCUS VUILLEMINII AND RHIZOPUS NIGRICANS.

In ammonification studies with soil microörganisms an incubation period of 7 days has been quite generally adopted. While this point has been treated to a considerable degree in investigations concerning bacteria, such has not been the case with regard to soil fungi. Waksman and Cook (16) in studies with *Mucor plumbeus, Penicillium sp.* and *Monilia sitophila* found that the most practical duration for incubation was 12 days. In the present investigation the organisms under observation were *Zygorrhyncus Vuilleminii* and *Rhizopus nigricans.* The soil was the same as that used in the previous work, namely Norfolk sandy loam; and the organic nitrogenous materials, dried blood and cottonseed meal were added in amounts equivalent to 155 mg. of nitrogen. As before, the soil was made up to optimum moisture content and sterilized in 200 c.c. Erlenmeyer flasks at 15 pounds pressure for 15 minutes. Upon cooling, the soil in each flask was inoculated with 1 c.c. of spore-suspension of the desired organism (prepared according to the method already described). The flasks were then incubated at 18 to 21° C. throughout the duration of the experiment.

TABLE VIII.

INCUBATION PERIOD OF ZYGORRHYNCUS VUILLEMINII. NORFOLK SANDY LOAM.

Lab. No.	Days	Organic Matter	H_2O c.c.	Mg. N.	Mg. N.	Av. Mg. N.	Increase over ch'k Mg. N.	Inc. over precd. day Mg. N.
91-92	Check	155 Mg. N. Dried Bld.	16.7	2.90	3.25	3.08	.....	....
1-2	1	"	"	3.20	3.30	3.25	0.17	0.17
5-6	2	"	"	3.53	3.50	3.52	0.44	0.27
7-8	3	"	"	3.47	3.50	3.49	0.41	—0.03
11-12	4	"	"	4.66	4.60	4.63	1.55	1.14
14-15	5	"	"	10.14	9.27	9.71	6.63	6.08
17-18	6	"	"	19.92	20.05	19.99	16.91	10.28
19-21	7	"	"	22.10	22.25	22.18	19.10	2.19
23-24	8	"	"	32.55	31.10	31.83	28.75	9.65
25-27	9	"	"	30.60	30.90	30.75	27.67	—1.08
29-30	10	"	"	33.70	33.68	33.69	30.61	2.94
31-33	11	"	"	34.50	34.48	34.49	31.41	0.80
35-36	12	"	"	36.17	35.85	36.01	32.93	1.52
38-39	13	"	"	35.99	36.10	36.05	32.97	0.04
41-42	14	"	"	36.10	36.00	36.05	32.97	0
43-45	15	"	"	37.00	37.78	37.39	[1]34.31	1.34

[1] Free ammonia liberated.

In Table VIII is presented the ammonia accumulated in the soil by *Zygorrhyncus Vuilleminii* acting on dried blood for 15 successive days. The column headed "increase over check in mg. N." which is graphically illustrated in figure 5 shows that there is practically no ammonification

until the 5th day, following which it takes place with increasing rapidity until the 8th day. After that time there is a slight increase (with one exception) until the 12th day, when a constant ensues. There is, then, good reason to believe that the maximum production of ammonia takes place

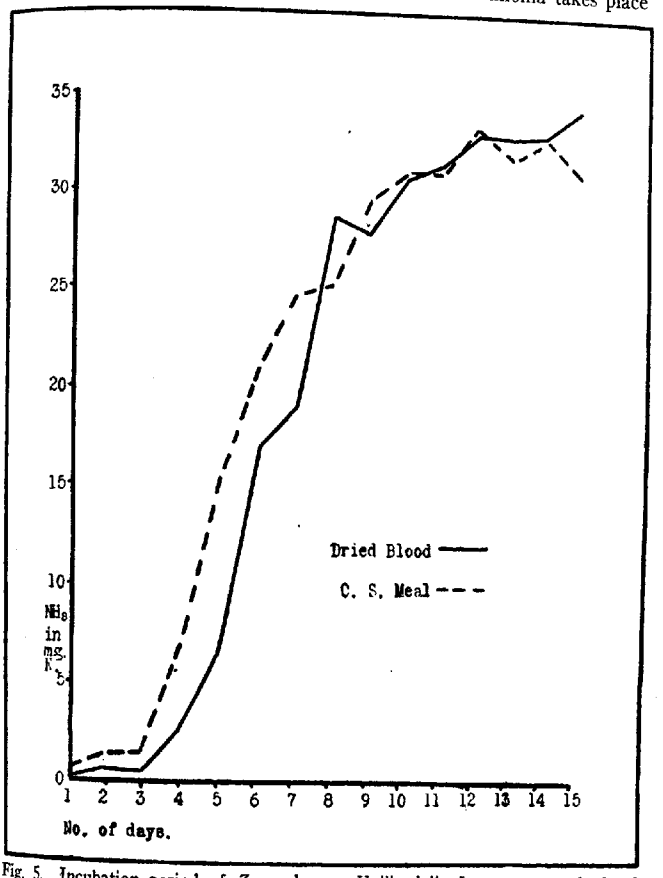

Fig. 5. Incubation period of *Zygorrhyncus Vuilleminii*. Increase over check of ammonia in mg. N.

between the 5th and 8th days. This might be interpreted as bearing out the theory advanced in discussing inoculation, namely that there is an increase in ammonia production with an increase in the number of spores, up to a certain point. (The number of spores may be seen to develop rapidly during the first week of growth.) On the other hand, an explanation of these results might depend, to even a greater extent, upon the metabolic processes involved.

It is interesting to note the daily increase in ammonia over the preceding day as shown in the last column of Table VIII and plotted in figure 6. It will be seen that there is a marked increase from the 4th to the 6th day, when the maximum gain is made. Thereafter there appears to be a singular fluctuation (up to the 12th day), where every other day marks a decided gain, the intervening days showing only slight increase. There has been an attempt made to correlate ammonia production with a biological stage of fungus, namely spore production and germination (15), but the above results do not seem to bear out such an hypothesis. The time elapsing between the germination of a spore of *Zygorrhyncus* and the appearance of sporangia on the mycelium developing from that spore is, roughly speaking, 3 days as determined by observation of the growth of the organisms in soil as well as in hanging drop preparations. Thus one would expect according to the theory just

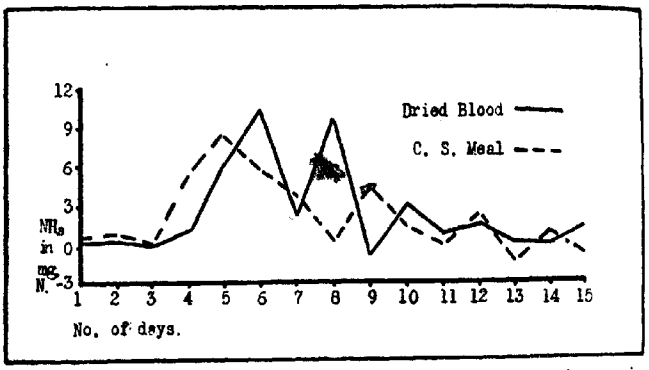

Fig. 6. Incubation period of *Zygorrhyncus Vuilleminii*. Daily increase in ammonia in mg. N.

set forth, that there would be a distinct increase in ammonia production every third day. But the data at hand points quite definitely to a sharp increase every *other* day. It appears to the writer that this phenomenon of fluctuation might more reasonably be interpreted in the light of the metabolic process of the fungus. In effect, ammonia accumulation, being a resultant of the concomitant factors of production and consumption, might fluctuate from day to day as a result of the predominance of first one and then the other of these two factors mentioned. Again, it will be noted that in one instance, namely, on the 9th day there was actually a decrease in ammonia as compared with the previous day. Undoubtedly, the production of spores is more or less continuous after the 4th day, therefore such a decrease seems to depend chiefly upon the metabolic processes rather than on any biological stage of a fungus.

In Table IX are recorded the results obtained in the ammonification of cottonseed meal by *Zygorrhyncus*. Upon directing attention to the column headed "increase over check in mg. N.," which is graphically illustrated in figure 5, it will be noted that there is very little ammonification up to the 4th day, after which there is a rapid increase to the 7th day. Subsequently there is a more variable increase up to the 12th day which marks the maximum. There is a decline after the 12th day. It will be observed that the maximum ammonia production occurs between the 4th and 9th days. In the last column of Table IX which represents the daily increase over the preceding day, which is graphically illustrated in figure 6, it will be seen that the greatest single increase occurred on the 5th day. Furthermore, the phenomenon of fluctuation, previously discussed, again becomes conspicuous after the 6th day. In other words, there is a sharp increase every other day followed by a less striking increase on the intervening days. On the 13th and 15th days, respectively, there was a decrease compared with the ammonia produced on the preceding day.

TABLE IX.

INCUBATION PERIOD OF ZYGORRHYNCUS VUILLEMINII.
NORFOLK SANDY LOAM.

Lab. No.	Days	Organic Matter	H_2O c.c.	Mg. N.	Mg. N.	Av. Mg. N.	Increase over ch'k Mg. N.	Inc. over preced. day Mg. N.
		155 Mg. N. Cot'nseed						
93-94	Check	Meal	20.5	3.85	3.75	3.80	.....	....
47-48	1	"	"	4.40	4.33	4.37	0.57	0.57
50-51	2	"	"	5.00	5.25	5.13	1.33	0.76
53-54	3	"	"	5.20	5.25	5.23	1.43	0.10
56-57	4	"	"	10.65	10.63	10.64	6.84	5.41
59-60	5	"	"	18.76	19.75	19.22	15.42	8.58
61-63	6	"	"	25.00	24.78	24.89	21.09	5.67
64-65	7	"	"	27.95	29.15	28.55	24.75	3.66
68-69	8	"	"	29.00	29.10	29.05	25.25	0.50
71-72	9	"	"	33.35	33.34	33.35	29.55	4.30
74-75	10	"	"	34.00	35.50	34.75	30.95	1.40
76-78	11	"	"	35.05	34.45	34.75	30.95	0
80-81	12	"	"	37.30	36.75	37.03	33.23	2.28
82-84	13	"	"	36.28	34.90	35.59	31.79	—1.44
85-86	14	"	"	37.30	35.90	36.60	32.80	1.01
88-89	15	"	"	36.55	32.68	34.98	31.18	—0.62

Comparing now the results obtained in the ammonification of dried blood (Table VIII), with that of cottonseed meal (Table IX) which may be seen quite readily in figure 5, it will be noted that the curves run practically parallel. Up to the 10th day (with one exception) the cottonseed meal is responsible for greater increases in ammonia than dried blood. This corroborates the conclusion arrived at in studies on inoculation, that for *Zygorrhyncus* cottonseed meal is a more acceptable source of food

than dried blood. Furthermore, it will be noted that cottonseed meal exhibits a fair production of ammonia on the 4th day, while this is delayed one day in the case of dried blood. From the 10th to the 14th day the curves are strikingly alike. The same is true of the results indicating the daily increase over the preceding day as shown in figure 6. It will be observed that the greatest single increase came on the 5th day in the case of cottonseed meal and on the 6th day with dried blood, which is the same as the relation which obtains between these two materials regarding the initial production of ammonia.

TABLE X.

INCUBATION PERIOD OF RHIZOPUS NIGRICANS.
NORFOLK SANDY LOAM.

Lab. No.	Days	Organic Matter	H_2O c.c.	Mg. N.	Mg. N.	Av. Mg. N.	Increase over ch'k Mg. N.	Inc. over precd. day Mg. N.
		155 Mg. N.						
142-143	Check	Dried Bld.	16.7	3.40	3.22	3.31	.....	....
144-145	1	"	"	3.80	3.39	3.60	0.31	0.31
146-147	2	"	"	5.15	5.11	5.13	1.82	1.51
148-149	3	"	"	20.81	20.66	20.73	17.42	15.60
150-151	4	"	"	27.74	27.15	27.43	24.12	6.70
152-153	5	"	"	31.06	31.00	31.03	28.72	4.60
154-155	6	"	"	29.74	34.31	32.02	29.71	0.99
156-157	7	"	"	42.50	45.31	43.95	40.64	10.93
158-159	8	"	"	35.31	35.42	35.36	32.05	—8.59
160-161	9	"	"	37.05	36.30	36.67	33.36	1.31
162-163	10	"	"	39.43	39.28	39.35	[1]36.04	[1]2.68
164-165	11	"	"	57.77	53.42	55.59	[1]52.28	[1]16.24

[1] Liberation of free ammonia.

Considering the influence of incubation period on the ammonification of dried blood by *Rhizopus nigricans* as shown in Table X and graphically illustrated in figure 7, it will be noted that there is practically no production of ammonia until the 3rd day, after which there is an increase up to the 7th day when a maximum is attained. After the 7th day there is a decline until free ammonia is liberated, as evidenced by odor and the litmus paper test. It was deemed unnecessary to continue the experiment for a longer period of time. Compared with *Zygorrhyncus*, this organism produces ammonia much more rapidly and attains its maximum in a shorter period of time. In hanging drop preparations the spores germinate within 24 hours. In considering the daily increase over the preceding day it will be observed that the maximum occurs on the 3rd day, or 3 days earlier than with *Zygorrhyncus*. From the 5th day on, the phenomenon of fluctuation previously noted, is apparent. As shown in figure 8, this is rather striking.

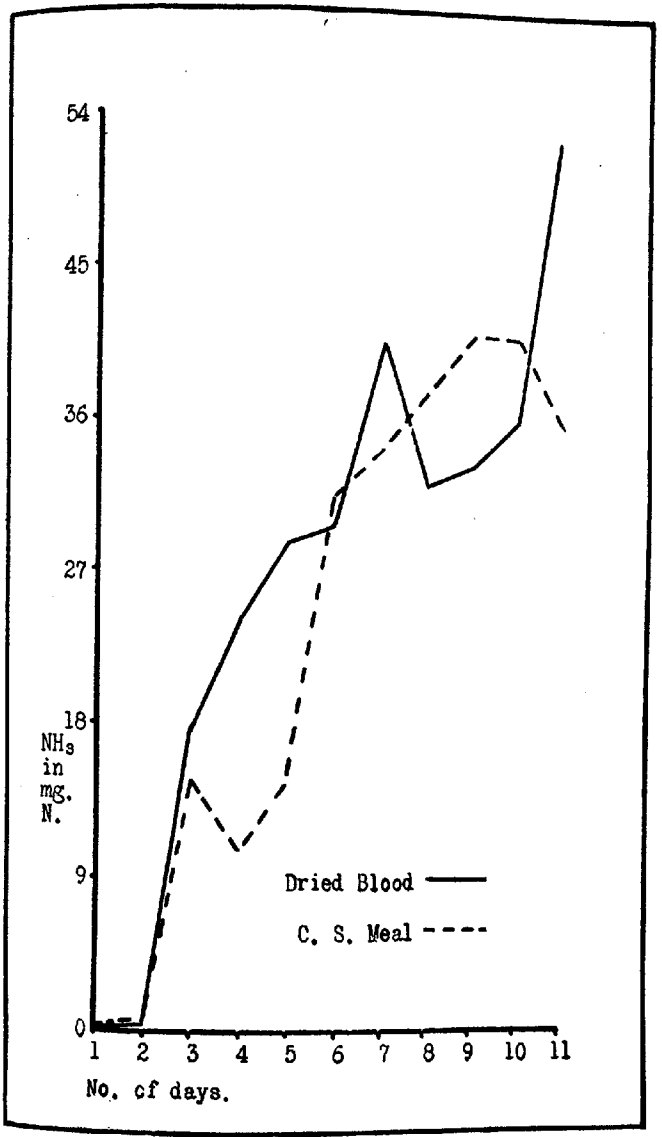

Fig. 7. Incubation of *Rhizopus nigricans*. Increase over check of ammonia in mg. N.

In Table XI and figure 7, may be seen the increase in ammonia accumulation where cottonseed meal is used as a source of organic nitrogenous matter. Again, as in the case of dried blood, the production of ammonia does not become vigorous until the 3rd day, and increases up to the 9th day when a maximum is attained. Thereafter a decline sets in, which occurs two days later than in the case of dried blood.

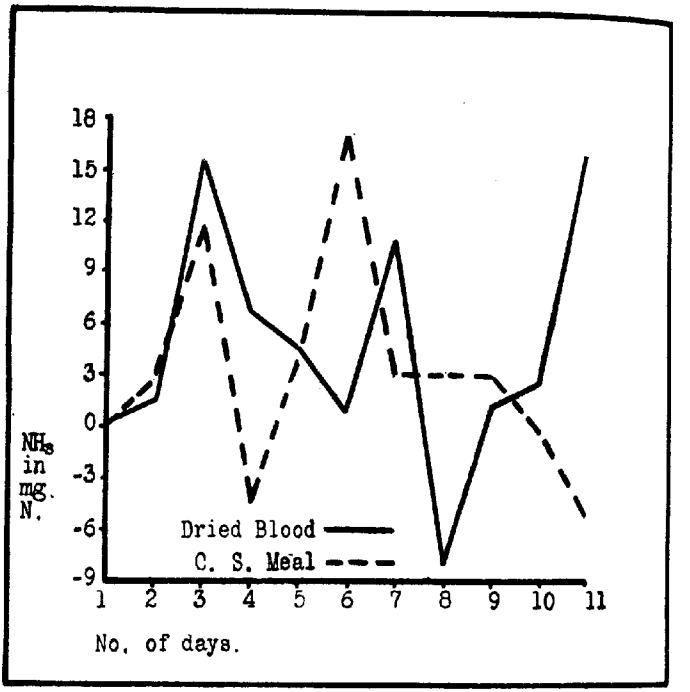

Fig. 8. Incubation period of *Rhizopus nigricans*. Daily increase in ammonia in mg. N.

With regard to the daily increase over the preceding day as shown in the last column of Table XI and in figure 8, a large increase occurs on the 3rd day, while a still larger appears on the 6th day. The fluctuation phenomenon is not as distinct as in previous cases, but the general tendency seems to point in the same direction.

To recapitulate, the maximum ammonification of dried blood and cottonseed meal by *Zygorrhyncus* occurs on the 12th day, while with *Rhizopus nigricans* the maximum ammonia accumulation with dried blood occurs on the 7th day and with cottonseed meal on the 9th day.

As in the case of inoculation, so likewise with incubation, it is essential to determine what period is best adapted to showing the difference between the ammonification of dried blood and of cottonseed meal.

TABLE XI.

INCUBATION PERIOD OF RHIZOPUS NIGRICANS. NORFOLK SANDY LOAM.

Lab. No.	Days	Organic Matter	H_2O c.c.	Mg. N.	Mg. N.	Av. Mg. N.	Increase over ch'k Mg. N.	Inc. over preced. day Mg. N.
		155 Mg. N. Cot'nseed						
165-166	Check	Meal	20.5	3.69	3.69	3.69	.....	.....
167-168	1	"	"	3.65	3.65	3.65	0	0
169-170	2	"	"	6.49	6.64	6.56	2.87	2.87
171-172	3	"	"	18.02	18.71	18.36	14.76	11.89
173-174	4	"	"	15.97	12.39	14.18	10.49	—4.27
175-176	5	"	"	21.69	14.46	18.07	14.38	3.89
177-178	6	"	"	35.27	35.07	35.17	31.48	17.10
179-180	7	"	"	38.52	38.08	38.30	34.61	3.13
181-182	8	"	"	41.46	41.40	41.43	37.70	3.09
183-184	9	"	"	44.91	44.18	44.59	40.90	3.20
185-186	10	"	"	44.45	44.49	44.47	40.78	—0.12
187-188	11	"	"	39.99	38.83	39.41	35.72	—5.06

Table XII has been prepared with this point in view, the figures recorded represent the remainder after subtracting the amount of ammonia accumulated with one organic matter from that accumulated by the other. It will be noted that with both fungi used, the 7-day incuba-

TABLE XII.

DIFFERENCES IN AMMONIFICATION BY SOIL FUNGI OF DRIED BLOOD COMPARED WITH COTTONSEED MEAL, AT 3, 7, 10 AND 15 DAYS' INCUBATION.

Organism	Differences between Ammonia accumulated in presence of Dried Blood and Cottonseed Meal (Increase over check av. mg. N.)		
	3 days	7 days	10 days
Zygorrhyncus Vuilleminii	1.02	5.65	0.34
Rhizopus nigricans	2.66	6.03	4.74

	Per cent of Maximum Ammonification					
	D. Bld.	C. S. M.	D. Bld.	C. S. M.	D. Bld.	C. S. M.
Zygorrhyncus Vuilleminii	1	4.2	57.9	75.1	93.9	84.0
Rhizopus nigricans	42.8	36.2	100.0	84.6	90.0	99.5

tion period showed the most striking differences. Furthermore it will be seen from the lower half of table XII that with regard to the percentage of maximum ammonification, the 3-day period cannot be considered adequate, while the 7-day period is not markedly inferior to the 10-day period. Since the 7-day incubation period involves less risk of loss of free ammonia from the soil as well as evaporation of moisture, and repre-

sents an economy of time, it is to be recommended as the most desirable incubation period for the soil fungi considered. From observation, it is also suggested that this would hold true for most other groups of soil fungi, with the exception of the important *Penicillia* and possibly some others.

SUMMARY.

Under the conditions of the experiment the following points may be noted in studies with *Penicillium sp.* 10, *Rhizopus nigricans, Zygorrhyncus Vuilleminii,* and *Rhizopus Oryzae,* in Norfolk sandy loam.

1. An increase in the number of fungi spores inoculated into the soil is responsible for a proportional increase in ammonia accumulation.

2. One c.c. of spore-suspension, all factors considered, is the most desirable quantity for inoculation in experiment with pure cultures of soil fungi.

3. Increasing the number of spores used in inoculation beyond a certain point does not further accentuate the difference between the ammonification of dried blood and cottonseed meal by these fungi.

4. Under the conditions of moisture and temperature employed, it appears that cottonseed meal is a more acceptable source of food than dried blood for the organisms studied.

5. With *Zygorrhyncus Vuilleminii* the maximum ammonia accumulation occurs on the 12th day with both kinds of organic matter. With dried blood *Rhizopus nigricans* yields the maximum on the 7th day and with cottonseed meal on the 9th day.

6. All factors considered, a 7-day incubation period may be recommended as most desirable for the study of soil fungi, other than those belonging to the Penicillium group.

7. There is a striking increase in ammonia production taking place every *other* day (after the first five days).

8. There is good reason to believe that the production of ammonia is dependent on the metabolic processes of the fungus rather than the biological stage of spore production and germination.

In conclusion it is a privilege to thank Dr. J. G. Lipman for his helpful suggestions ever at the writer's disposal.

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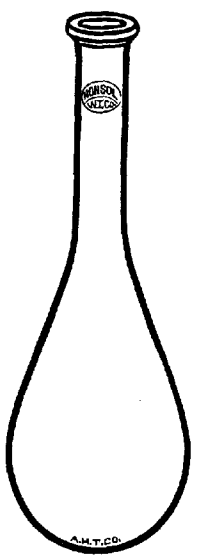

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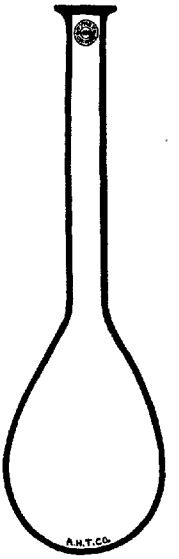

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